THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME VI JULY, 1930 NUMBER 4

TISSUE REACTIONS IN RABBITS FOLLOWING INTRAVENOUS INJECTION OF BACTERIA *

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Reference has been made in a recent paper [1] to the peculiar reaction in rabbit tissues to repeated injections of non-hemolytic (gamma type) streptococci. A preliminary report [2] of the general distribution and histology of these lesions has been made. In general, the lesions are characterized by the mobilization, in various organs of the body, of large mononuclear cells which appear to be monocytes, according to supravital classification, and which quite frequently lead to the formation of giant cells of the Langhans' type. Because of the unusual and peculiar lesions, because of the type of cell called forth and because of the nature of the antigen which results in this response, the observation would appear to be of interest to the pathologist, cytologist and immunologist.

The present paper deals with the methods used to produce the lesions and with a detailed description of the histological changes. Different methods of injection have been tried, various organisms have been tested for their ability to produce the changes, and other animals, namely, guinea pigs, have been utilized.

EXPERIMENTAL

Intravenous Injection

Method: Injections were made into the ear veins of rabbits (average weight 1800 gm.) at intervals of four or five days. With living 24 or 48 hour broth cultures of the gamma type streptococci the initial dose was 1 cc., and with dead

* Read before the American Association of Pathologists and Bacteriologists, May 1, 1928, Washington, D.C.

Received for publication March 1, 1930.

cultures 5 cc. For subsequent injections the former was increased to 5 cc., then to 10 cc., and the latter to 10 cc. With the control injections of Berkefeld filtrates of broth cultures and with the injection of heat-killed filtrates of other organisms, the dosage and time intervals were the same as with the dead streptococci, except with *B. coli* and *B. tuberculosis* where the initial dose was 1 cc. The tubercle bacilli were grown for three weeks on coagulated egg medium and a heavy saline suspension was used. Cultures were usually killed by heating at 60° C for 20 minutes and were always tested before injection by subculturing to suitable media. As a rule, a minimum of 6 doses was given. The guinea pigs were usually injected through one of the superficial leg veins; the initial dose was 0.5 cc. and subsequent doses increased to a maximum of 5 cc., although frequently only 2 cc. could be injected due to the difficulty of injection. Supravital stains and total leucocyte counts of the circulating blood were made on many of the animals before and after the injections.

All animals, whenever possible, were killed painlessly with ether and autopsied immediately. Fresh smears from the cut surfaces of the organs were frequently taken and stained by the supravital technique. The tissues were fixed in Zenker's solution and in 10 per cent formalin. Routine sections were made from the Zenker-fixed tissue in paraffin and stained with eosin and methylene blue. When advisable, sections were stained by the Gram Weigert method and by Foot and Mènard's [3] modification of Hortega's silver carbonate method.

The majority of animals that received injections of killed bacteria showed no ill effects; there were no pronounced losses in body weight or of appetite. A few of the animals died during the course of injection; in some no apparent cause could be found, others probably died from the effects of endotoxins, such as those of *B. coli*, and in others the characteristic lesions in the lungs were present to such a degree as to produce pulmonary circulatory changes of sufficient gravity to be considered a contributory cause of death. Total white blood corpuscle counts and supravitally stained (neutral red) differential counts of the circulating blood were made before and after injections on many of the animals. The majority of these rabbits were those that were to receive or had received injections of killed non-hemolytic streptococci. As a rule, there was a moderate rise in total count with a moderate to marked increase in the absolute number of monocytes and a corresponding decrease in the number of lymphocytes. Some rabbits showed no appreciable changes. The maximum percentage of monocytes was 39 per cent (Rabbit No. 25, 24 hours after the ninth injection), and many of the bloods gave figures higher than 20 per cent, as compared with a maximum of 10 or 12 per cent before injection. The results on two groups of rabbits, other than those receiving the non-hemolytic streptococci, are of interest. Two rabbits, Nos. 27 and 28, which were injected

with filtrates from broth cultures of non-hemolytic streptococci, showed moderate increases in monocytes (22 and 19 per cent, respectively) on the day following the fifth injection with a gradual decline to normal, subsequently. Two other rabbits, Nos. 85 and 86, showed no abnormality of the circulating blood leucocytes on the day following the sixth injection of massive doses of dead tubercle bacilli.

The sera of rabbits that had received six or more injections of living non-hemolytic streptococci usually showed definite macroscopic agglutination against the homologous organisms in 1:1280 or 1:2560 dilutions.

At autopsy the only abnormalities noted in animals that had received a sufficient number of injections of dead organisms to result in marked microscopic lesions, were failure of the lungs to collapse on opening the pleural cavities, with an increase in consistency on sectioning and moderate to marked increase in the size of the spleen.

The variations in reaction of the same species of animal to different organisms, of a different species of animal to the same organism, and so forth, are of interest. In order to be able to describe accurately such variations it will be necessary to give a detailed histological description of the characteristic lesions produced in the rabbit following intravenous injection.

Lungs: After the second or third injection the most characteristic changes are focal collections of cells about large and small veins. These foci consist of cells which appear to be lymphocytes and resemble those of the peribronchial lymph nodules which are normally present, except that the cells are fewer in number and appear to be less mature; mitoses are fairly common. At this time the alveolar walls show some increase in cells which are apparently normal in type. After the third or fourth injections, obvious venous lesions make their appearance. These consist of collections of cells in the loose subendothelial connective tissue of the intima, and are usually in close relation to the perivascular foci of lymphoid cells. The cells in the earliest lesions appear to be of the lymphocytic series, but, as the lesions progress the cells have the characteristic appearance of monocytes, according to ordinary staining and to the supravital classification, and giant cells of the Langhans' type are often present. The infiltration may proceed to complete occlusion of the lumen, particularly in the smaller veins, and this results in a lesion very similar to a tubercle. The larger veins are never completely occluded, but frequently show a marked infiltration at both sides of the points of entrance of smaller veins, suggesting that the cells of the exudate have pushed their way up underneath the endothelium from the smaller into the larger vessel. In all instances the lining endothelium is intact, and true thrombi are only rarely observed. Lesions of this sort are never found in the arteries or arterioles, except for an occasional tiny focus, consisting of

three or four cells of the lymphoid type, beneath the endothelium of the larger arteries. The changes which occur in the capillaries of the alveolar walls are marked, but difficult to follow. There is a thickening of the alveolar walls, and in various areas the alveoli are obliterated. Fairly large foci of lymphoid cells are found and smaller foci of the large mononuclear cells and giant cells. The latter appear to originate from lesions in or about the smaller venules and capillaries, similar to those found in the veins. Large phagocytic mononuclear cells, some of which are similar in staining reaction to those in the veins and alveolar walls, are found free in the alveoli. All the normal alveolar structure may disappear and be replaced by areas of true consolidation. Smears from the cut surface of such lungs, stained by the supravital method, show a predominance of cells classified as monocytes. The giant cells have the typical monocytic "rosette." In sections from lungs obtained within a few hours after injection and stained by the Gram Weigert method, the organisms can be easily demonstrated. They are phagocytized most often by mononuclear cells which are usually within, or in close relation to, the capillaries of the alveolar wall. Occasional polymorphonuclear cells are seen which contain the bacteria. The organisms have never been observed in the multinuclear cells which are found in the characteristic subendothelial vascular lesion or in those free in the alveoli. Many more bacteria are found in the lungs than in the liver and spleen. Animals killed one day, or longer, after the last injection show few, if any, organisms.

Liver: After two or three intravenous injections small islands of lymphoid cells are found in the sinusoids of the liver lobules, chiefly at the periphery. Mononuclear cells with large pale ovoid nuclei appear in these islands and eventually giant cells are formed. There is a generalized increase of cells between the liver columns, involving the cells of the sinusoidal endothelium and connective tissue cells. The columns of parenchymal cells are narrowed, particularly at the periphery of the lobule, but there is no apparent necrosis. Occasionally tiny projections into the lumina of the larger hepatic veins are observed which are similar to the very early lesions in the pulmonary veins. Supravitally stained smears of freshly cut sections show many mononuclear and multinuclear cells which are classified as monocytes. In Gram Weigert stained paraffin sections from rabbits killed within a few hours after the last injection, many bacteria are seen phagocytized by the normal appearing endothelial (Kupffer) cells of the sinusoids; none are found in the large mononuclear or multinuclear cells.

Spleen: After the third intravenous injection clumps of large mononuclear cells are seen in the splenic pulp adjacent to the terminal veins or splenic sinuses. Eventually these increase in size, and frequently such collections contain numerous giant cells. Oftentimes they are found adjacent to the splenic nodules. In the advanced form they closely resemble miliary tubercles. The germinative centers of the splenic nodules are usually extremely active, showing a large percentage of pale immature cells and many mitoses. In Gram Weigert stained sections, the organisms are found in the relatively large phagocytic cells ("splenic cells") of the normal spleen. As these cells phagocytize leucocytes, one cannot say whether the bacteria are taken up first by the splenic cells or whether they have been phagocytized primarily by phagocytic leucocytes. Fewer organisms are found in the spleen than in the lung and liver from the same animal.

Kidney: Giant cells are sometimes found in the glomerular tufts.

Pancreas: Negative.

Adrenal: Giant cells are rarely found in the *zona fasciculata* between the columns of parenchymal cells.

Small Intestine: Negative.

Mesenteric Lymph Nodes: Negative, except for hyperplasia.

Heart: In several animals subendothelial lesions similar to, but less pronounced than, those found in the lungs have been observed in one or more of the medium-sized veins draining the myocardium.

Bone Marrow: The bone marrow, as a rule, is hyperplastic, without any particular change in cell types. Rarely true giant cells, as distinguished from megakareocytes, are found.

Skeletal Muscle: Negative.

Brain: Negative.

A list of the animals injected intravenously is given in Table I. As the characteristic lesions were found most commonly in the lungs, liver and spleen, the results of microscopic examination of sections of these organs have been used to determine whether or not the injections produced the changes in question.

The lesions described above were first noted [1] in rabbits that had been injected with living cultures of non-hemolytic (gamma type) streptococci for the purpose of obtaining agglutinating sera. One strain ("Small A") was chosen as representative of those organisms capable of producing the reaction in the rabbit and an attempt was made to obtain further information by varying certain factors. Heat-killed (60° C for 20 minutes or 100° C for 20 minutes) cultures were found to produce the same lesions as the living cultures, except, of course, for the occasional acute vegetative endocarditis found in animals injected with the latter. Provided the animal was killed within five or six days after the last injection, definite changes in the lungs and liver were always found following two intravenous injections, and marked changes occurred after five or more doses. Two rabbits, Nos. 1 and 44, that were killed nine and thirty days respectively after the last injection, and that had received a sufficient number of injections to produce, ordinarily, pronounced lesions showed no histological changes of note. Two rabbits, Nos. 43 and 49, that received 16 and 12 injections of killed organisms and were killed one and five days, respectively, after the last injection, had definite lesions in the lungs and liver, but they were much less marked than those found in animals after five or six doses, and the presence of a certain amount of fibrosis gave evidence of regression and repair. Two rabbits, Nos. 27 and 28, that received the Berkefeld

TABLE I

Animal		Organism	Culture	Doses	Fate of animal	Time after last injection	Tissue reactions		
No.	Kind						Lung	Liver	Spleen
1	Rabbit	Streptococcus, gamma type, "Small A"	Living	6	Killed	9 days	–	o	o
2	"	" " " " "	"	5	Died	24 hours	++++	++++	++
23	"	" " " " "	"	6	"	24 "	++++	++++	++++
24	"	" " " " "	"	9	Killed	24 "	++++	+++	++++
25	"	" " " " "	Dead	9	"	3 days	+++	++++	+++
26	"	" " " " "	"	6	Died	5 hours	++++	++++	+++
27	"	Berkefeld filtrate of "Small A" culture		9	Killed	4 days	o	o	o
28	"	" " " " " "		6	Died	3 "	o	o	o
29	"	Streptococcus, gamma type, "Small A"	Dead	2	Killed	4 "	+	+	+
30	"	" " " " "	"	2	"	4 "	+	+	+
31	"	" " " " "	"	3	"	5 "	+	++	++
32	"	" " " " "	"	3	"	5 "	+++	+++	++
33	"	" " " " "	"	4	"	4 "	+++	++	+++
34	"	" " " " "	"	4	"	4 "	++++	++++	++++
43	"	" " " " "	"	16	Died	24 hours	+, old	+, old	–
44	"	" " " " "	"	6	Killed	30 days	o	o	o
45	"	" " " " "	"	4	Died	2 "	++	+	+
48	"	" " " " "	Dead, 100°C	6	Killed	4 "	++++	++++	++
49	"	" " " " "	Dead	12	"	5 "	++, old	++, old	++
50	"	" " " " "	"	6	"	2 "	++++	++++	–
53	"	" " " " "	"	8	"	24 hours	+++	++++	–
55	"	" " " " "	"	8	"	24 "	++++	++++	++

65	Rabbit	Streptococcus, gamma type, "Small A"	Dead	3	Killed	3 days	++	±	±
77	"	" " " " "	"	6	Died	2 "	+++	+++	+++
78	"	" " " " "	"	8	"	4 "	+++	++++	+++
80	Guinea pig	" " " " "	"	6	Killed	4 "	+?	±	±
82	Rabbit	Streptococcus ("Small A") + carmine	"	6	Died	6 "	++	+++	–
83	"	" " " "	"	6	"	2 "	+++	+++	–
84	"	" " " "	"	6	Killed	4 "	++	++	+++
87	Guinea pig	Streptococcus, gamma type, "Small A"	"	6	"	3 "	+?	±	±
88	" "	" " " " "	"	6	"	3 "	+?	±	o
5	Rabbit	Streptococcus, gamma type, "Small B"	Living	6	Killed	15 days	–	+, old	–
9	"	" " " "Birk. 2"	"	6	Died	6 "	++++	+++	++
12	"	" " " "R.F.T. 4"	"	5	Killed	4 "	o	o	o
22	"	" " " "N.T. 2–8"	"	12	"	11 "	o	o	o
46	"	" " " "N.T. 21"	Dead	6	"	2 "	++++	++++	++++
47	"	" " " "N.T. 16"	"	6	Died	2 "	+++	++++	o
35	"	Diplococcus, green-producing, "B–26–27"	"	6	Killed	4 "	o	±	±
36	"	" " " "	"	6	"	4 "	±	o	o
38	"	Streptococcus scarlatinae, "D I"	"	6	"	4 "	++++	++	+
59	"	Streptococcus hemolyticus	"	6	"	5 "	+	+	+
60	"	" "	"	6	"	2 "	++	+++	+++
63	"	Streptococcus viridans	"	6	"	2 "	±	±	+
64	"	" "	"	7	"	30 min.	o	o	±
58	"	Bacillus coli	"	6	"	4 days	++++	++++	++++
62	"	" "	"	4	Died	24 hours	++	++++	+++
85	"	Bacillus tuberculosis, bovine	"	6	Killed	2 days	++++	++	++
86	"	" " "	"	6	"	2 "	+++	+	+

– = not done, o = negative, ± = questionable, + = very slight, ++ = slight, +++ = moderate and ++++ = marked.

filtrate from killed cultures identical with those used to inject two rabbits, Nos. 25 and 26 in which typical reactions were found, showed no demonstrable lesions. Three rabbits, Nos. 82, 83 and 84, that, after their fifth dose of organisms, received daily doses of lithium carmine up to and past the sixth dose of bacteria, showed lesions which were less marked than those usually found at that period. The distribution of carmine, together with its significance, will be discussed in a subsequent paper.

Three guinea pigs, Nos. 80, 87 and 88, were successfully injected intravenously with killed cultures of "Small A." The only lesions of any significance were found in the lungs. The majority of small and medium-sized veins were surrounded by collections of lymphoid cells similar to those found in the early changes in rabbits. No abnormal cells were observed within the vessels, and, neither cells of the monocytic type nor giant cells could be demonstrated. Sections of the livers and spleens showed nothing remarkable. Two of the three sections of the adrenals showed large areas of necrosis resembling infarcts. One of these showed an acute inflammatory reaction, while the others showed merely necrosis.

Rabbits were injected intravenously with cultures of six other strains of non-hemolytic (gamma type) streptococci. Three of these strains ("Birk. 2," "N.T. 21," and "N.T. 16") produced typical reactions in the lungs, livers and spleens. The rabbits that received two other strains ("Small B," and "N.T. 2–8") were killed so long after the last injection, fifteen and eleven days respectively, that one would not expect to find any lesions, and none were found. The one other strain ("R.F.T. 4") failed to have any effect.

A green-producing diplococcus ("B–26–27"), isolated from the stool of a case of chronic ulcerative colitis, and a strain of *Streptococcus viridans*, isolated from the blood of a case of bacterial endocarditis, were injected without effect. The latter grew so poorly that the result is hardly comparable because of the relatively small number of organisms injected. With *Streptococcus scarlatinae* ("Dick I") definite lesions were obtained, most marked in the lungs, whereas with a strain of *Streptococcus hemolyticus* the changes in the liver and the spleen were the most prominent.

The injection of killed cultures of *B. coli* resulted in changes identical with those previously described. The lesions in the liver appeared to be more marked than those in the lungs, as compared

with the same lesions following the injection of gamma type streptococci.

Two rabbits, Nos. 85 and 86, were injected with suspensions of dead bovine tubercle bacilli. In these animals the most pronounced lesions were in the lungs, although a few giant cells were found in the spleens and a moderate number in the livers. The pulmonary lesions were somewhat different from those previously described. There were many foci of large pale staining mononuclear cells and giant cells, surrounded by zones of lymphoid cells. These areas had apparently arisen from lesions in small veins or capillaries and looked like true miliary tubercles. Subendothelial lesions in the larger veins were present, but were not as prominent as in other rabbits. In sections stained to demonstrate the tubercle bacilli, the organisms were found in the lungs in the giant cells and in the mononuclear cells, both of the tubercle-like foci of the subendothelial lesions and of those occurring singly in alveolar walls. In the livers the bacilli were found occasionally in the giant cells and in the small groups of mononuclear cells which were apparently the forerunners of the giant cells. The giant cells and the large pale mononuclear cells in the spleen contained a few acid-fast rods and granules. Intradermal tuberculin tests in both animals, before and after the series of injections, were negative.

Through the kindness of Dr. Gulli Lindh Muller, sections of organs of rabbits that had received repeated injections of colloidal substances, such as India ink and collargol, were made available for study. Furthermore, sections from rabbits that had received repeated intravenous injections of collargol and that had been previously described by one of the authors in conjunction with Stewart [4] were restudied. In rabbits that had received 6 to 12 injections, the lesions in the livers and spleens were practically identical with those found following the injection of killed bacteria and the distribution of pigment was the same as that of the dead tubercle bacilli. There were many pigmented giant cells in the livers, chiefly in the sinusoids at the periphery of the hepatic lobules, but also at the edges of the periportal spaces. Furthermore, the majority of endothelial cells lining the sinusoids contained pigment. In the spleens the germinal centers were hyperactive; and, amongst the lymphoid cells at the periphery of the germinal centers, in the splenic pulp and, occasionally, free in the sinusoids, were numerous pigmented mononuclear

cells and giant cells. The lesions in the lungs were much less marked than those following the injection of bacteria, but nevertheless identical changes could be demonstrated. The pigmented mononuclears and giant cells were found chiefly in relation to the capillaries in the alveolar walls and to the peribronchial and perivascular collections of lymphoid cells. Definite subendothelial lesions consisting chiefly of pigmented macrophages could be demonstrated in one or more of the medium-sized veins of each specimen, but they were relatively slight as compared with those previously described. Little or no pigment was found in the endothelial cells of the capillaries and larger blood vessels.

Intraperitoneal Injection

Two rabbits, Nos. 39 and 40, were injected intraperitoneally with killed cultures of gamma type streptococci ("Small A"). The initial dose was 5 cc. and this was increased to 10 cc., then to 20 cc., with injections at four or five-day intervals. One rabbit, No. 39, developed "snuffles" and died on the day following the fifth injection; there was a marked loss of weight. The other rabbit, No. 40, which showed a moderate loss of weight, was killed on the second day following the sixth injection. The findings at autopsy were identical in both instances. All the organs in the peritoneal cavity were bound together and to various portions of the parietal peritoneum by friable fibrinous adhesions. There was a marked general thickening of the peritoneum in which were many pin-point to pin-head-sized pale yellow foci which contained a firm pale yellow exudate. The omentum was greatly thickened and contained a moderate number of these foci. The mesenteric lymph nodes were somewhat enlarged. There was no free fluid. All organs, on section, proved negative except for the thickened visceral peritoneum about the abdominal organs. Cultures from the peritoneal cavity and heart's blood yielded no growth. Smears taken from the peritoneum showed a marked increase in the number of cells with a predominance of small and large mononuclear cells.

The lungs, liver and spleen, on microscopic examination, showed none of the changes observed following intravenous injection; in fact, the only deviation from normal histology was observed in the peritoneum. In sections from the omentum a marked thickening

composed of very active atypical granulation tissue was observed. There were many engorged young blood vessels and numerous fibroblasts undergoing mitosis. Many of the larger blood vessels were surrounded by collections of lymphoid cells, but there were no subendothelial lesions. Scattered throughout the young connective tissue were a few polymorphonuclear leucocytes and many lymphocytes and plasma cells. In addition there were many large mononuclear cells, some of which were undergoing mitotic division, and in certain areas there were a few giant cells of the Langhans' type. The pale yellow foci, observed grossly, consisted of areas containing many of the cells, mentioned above, packed closely together. The centers of such foci usually showed necrosis with an accompanying predominance of polymorphonuclear leucocytes.

Subcutaneous Injection

Subcutaneous injection of 1 cc. of a killed culture of a non-hemolytic streptococcus ("Small A") into the anterior abdominal wall of a normal rabbit resulted, after twenty-four hours, in an area of marked brawny edema measuring 20 by 30 mm., with slight erythema. After forty-eight hours the animal was anesthetized and the lesion removed. Microscopically the lesion showed a small abscess surrounded by a diffuse acute inflammatory reaction extending from the corium down through the muscle layers, with necrosis of many fibers of the latter. When stained by the Gram Weigert method a moderate number of the polymorphonuclear leucocytes were found to contain bacteria.

After six intravenous injections the same rabbit was injected again, subcutaneously. There was practically no induration after twenty-four hours, and after forty-eight hours only a small nodule at the site of injection remained. This lesion was also removed on the second day and on examination showed a rather definitely localized abscess with only a moderate amount of surrounding acute inflammatory reaction. The cells in the abscess were predominantly polymorphonuclear leucocytes, but there were many mononuclear cells. The former contained many phagocytized bacteria, many more apparently than those in the previous lesion.

Two other rabbits injected subcutaneously, subsequent to intravenous injections, showed local swellings without surrounding

edema. One of these lesions was removed on the seventh day and showed a walled-off abscess without any surrounding reaction. The cells in the abscess were predominantly mononuclears, but the phagocytized bacteria which still persisted were found in polymorphonuclear cells.

Intradermal Injection

Several rabbits, either normal or previously injected intravenously, were injected with 0.2 cc. of a heated culture of a non-hemolytic streptococcus ("Small A") intradermally. All showed after twenty-four and forty-eight hours a tiny yellow nodule at the site of injection, without any surrounding erythema.

Discussion

When the lesions, which have been described, were first observed in tissues from rabbits that had received injections of living non-hemolytic (gamma type) streptococci, it was thought that they represented a specific reaction to infection with the organism used. Subsequent work, however, showed clearly that identical changes resulted following the injection of dead organisms of various sorts and, to a less degree, particularly in the lung, to injections of particulate matter, such as India ink and collargol. It would appear that these lesions represent the non-specific reaction of the rabbit in the process of disposing of certain foreign materials injected into the blood stream. That the changes are temporary and result in no serious permanent damage is shown by the essentially negative findings in animals killed a week or more following the last injection. The ability of the rabbits, at least in the case of killed bacteria, to dispose of the injected material, following many injections, without requiring as marked a degree of tissue reaction as at first, is evidenced by the relatively mild lesions in animals that received as many as 12 to 16 injections. These facts would seem to indicate that the primary response is that of a normal animal, rather than that of an immunized animal, or of one with its tissue reactions altered due to repeated injections resulting in allergic manifestations. The mononuclear cell, characteristic of the lesions, is intimately associated with tissue changes such as those of tuberculosis which are usually considered to be of an allergic nature and the time intervals

of the injections of the rabbits are such that one might expect a resulting allergic state. Intradermal and subcutaneous test injections, however, in rabbits that had received previous intravenous injections showed decreased, rather than increased, tissue reactions, and experiments such as those of Zinsser [5] and Swift and Derick,[6] have shown that allergy to bacteria or bacterial products usually does not follow intravenous injection, presumably because of the absence of focal lesions. A recent article by Ehrich,[7] in which identical subendothelial lesions are described in rabbits three to seven days following the intravenous injection of relatively large single doses of killed staphylococci, is additional proof that the reaction is that of a normal animal.

Monocytosis has been observed in experimental animals, and, in some instances, has been associated with certain infections such as tuberculosis (Sabin and Doan [8] and Camp, Luton, Tompkins and Cunningham [9]), Virus III disease in rabbits (Pearce and Casey [10]) and a disease in rabbits produced by *B. monocytogenes* (Murray, Webb and Swann [11]). Similar increases, however, have followed the injection into rabbits of acid-fast bacilli, such as, *B. smegmatis* (Jones and Tirrill [12]), *B. phlei* (McJunkin [13]) and *B. leprae* (Schwartz and Cunningham [14]), which are ordinarily considered non-pathogenic, the injection of phosphatide fractions of human tubercle bacilli (Sabin and Doan [15]) and the injection of dead cultures of *B. monocytogenes* (Witts [16]). Furthermore, a marked periodic increase in circulating monocytes following the injection of colloidal substances, including protective colloids, has been noted by Simpson.[17] Lawrence, Tompkins and Cunningham [18] were able to produce lesions resembling tuberculosis by the subcutaneous injection of many supposedly inert substances. Ray and Simpson [19] injected guinea pigs subcutaneously and into the lungs with living tubercle, grass and colon bacilli and with defatted bacilli or with lipins from the same organisms. Identical tubercle-like lesions were obtained with all these organisms or materials, with the exception that caseation was observed only with living tubercle bacilli. Very recently Wright [20] has described the local production of monocytes and Langhans' giant cells following the subcutaneous injection of various non-irritating gases, such as oxygen, nitrogen and carbon dioxide. A consideration of these observations would lead one to believe that such increases in monocytes are due to the presence in the blood stream,

or locally, of certain foreign materials not necessarily toxic, rather than to infection. The rise of circulating monocytes following the intravenous injection of non-hemolytic (gamma type) streptococci is undoubtedly a reaction of similar nature.

The reactions in the liver and spleen which have been described in this paper, are practically identical histologically with those seen following the intravenous injection of lamp black, India ink, collargol, colloidal iron, and so forth. Such lesions have been accurately pictured in papers by McJunkin,[21] Foot,[22] Polson [23] and Stewart and Parker.[4] Following the intravenous injection of minute daily doses of silica sol in rabbits, Gye and Purdy [24] have described marked endothelial changes in the liver and spleen and some in the glomeruli, lungs, cortices of the adrenals, and reticulum of the lymph nodes, which consisted of compact masses of endothelial cells with occasional giant cells and which were ascribed to the toxic action of the silicon ion. The pulmonary subendothelial lesions in the small veins and venules are quite inconspicuous following such injections, and have usually failed to attract attention. Foot [22] noted in splenectomized animals some thickening of the alveolar walls which he considered to be due to the swelling of the capillary endothelium and, to a lesser degree, to the accumulation of mesenchymoid cells in the interstitial tissue; frequently cells resembling tumor giant cells were present.

Many contributions have been made relative to the tissue changes following the intravenous injection of bacteria or bacterial products. The older papers deal, chiefly, with the lesions following the intravenous injection of dead tubercle bacilli or their products (Prudden and Hodenpyl,[25] Vissman,[26] Klett,[27] de Giaxa,[28] Morse and Stott [29] and Jaffé [30]). In general, using dead tubercle bacilli, lesions were found in the lungs, liver and spleen which resembled true tubercles except for the absence of caseation. Identical lesions were obtained, however, with extracts of tubercle bacilli,[27, 28, 29] and with other organic materials,[30] and the consensus of opinion seemed to indicate that the active principle was some toxic substance normally contained in the tubercle bacillus. No mention is made of subendothelial venous lesions in the lungs, but Vissman [26] noted that the *arterial* walls were thickened and infiltrated with round cells, in places so marked that the lumina were nearly obliterated, and Morse and Stott [29] called attention to the fact that the endothelial cells of the

capillaries in the lungs proliferated to such an extent that there was obliteration of the lumina and that the normal endothelial tubes were changed to syncytial cords. The more recent papers are concerned, chiefly, with a comparison of the tissue reactions in normal and sensitized animals. Following the intravenous injection of hen's red blood corpuscles in guinea pigs, Oeller [31] observed an increase in the peri-arterial collections of lymphoid cells in the lungs and spleen, desquamation of the capillary endothelium of the lungs and spleen to form monocytes, and marked endothelial changes in the arteries and veins. He interpreted these lesions as toxic changes secondary to the phagocytosis and destruction of the foreign corpuscles and noted that they appeared sooner in animals that had received repeated injections. Following repeated intravenous injections of dead or living *B. coli* in rabbits, Siegmund [32] noted endothelial changes in the liver, spleen, lungs and marrow. These consisted, mainly, in proliferative changes and in the formation of intravascular thrombi composed of large pale cells with vesicular nuclei, and subintimal and subendothelial reactions to these thrombi were often present. Domagk [33] injected specific shocking doses into mice rendered anaphylactic to live or dead staphylococci, streptococci, *B. coli* or to serum, and reported marked endothelial changes in the liver and lungs. In the liver, cell knots were formed in the sinusoids resulting in compression of the parenchyma, and, in the lung, the proliferative changes resulted in compression of the capillaries. The latter changes were considered to be sufficient to interfere mechanically with respiration and to produce death. After single and repeated injections of killed staphylococci injected intravenously in rabbits, Ehrich [7] has described marked mesenchymal reactions which in distribution and in composition are identical with those described in this paper and which he attributed to the primary reaction of normal rabbits to the bacteria or their products. Seemann,[34] Gerlach and Finkeldey [35] and Gerlach and Haase,[36] however, using essentially the same methods, have failed to observe, particularly in the lungs, any marked histological changes which they consider as pathognomonic.

It is apparent that tissue changes closely resembling those described in this paper have been observed in experimental animals following the intravenous injection of any of a variety of substances, and that such changes represent a non-specific reaction of the ani-

mal, as a whole, to some foreign material. That the reaction is not one of sensitization or of immunization is further substantiated by the observation that apparently identical lesions are produced by a substance, silica sol, which contains no nitrogen. The question arises as to whether such changes represent proliferative reactions, secondary to phagocytosis, on the part of the various cells of the body which belong to the reticulo-endothelial system, whether they are produced as a result of the toxic action of the substances injected or products formed in the body from such substance, or whether they arise from a combination of the two.

In general, the sinusoidal endothelium of the liver and spleen is considered to be the most important tissue for removing foreign substances from the blood stream. The capillary endothelium of the lungs has practically no phagocytic ability. Several investigators, however, have reported findings which indicate that the tissues of the lungs, at least, in certain animals, do exhibit marked phagocytic properties. Wyssokowitsch,[37] following the intravenous injection of bacteria, obtained numerous organisms from the lung at a time when blood cultures were negative, and Werigo [38] reported the same finding. Drinker and Shaw [39] injected finely dispersed manganese dioxide intravenously into cats and after one hour were able to recover from the lungs 47 per cent of the material injected. Polson [23] recovered large amounts of iron from the lungs of rabbits following intravenous injection of colloidal iron, but attributed the result to the presence in the blood vessels of emboli containing large amounts of the colloid. Hopkins and Parker [40] injected relatively large doses of living streptococci intravenously in cats and rabbits and were usually able to recover more organisms from the lungs than from any of the other organs. Mole [41] studied the reticulo-endothelial system of the rabbit by observing the phagocytosis of stained foreign erythrocytes and called attention to the importance of the lung in this system of the rabbit. Recent work in this laboratory has shown that, following the intravenous injection of large doses of bacteria, there is a marked increase of polymorphonuclear leucocytes in the lungs. Such an increase in cells, many of which, under these conditions, usually contain large numbers of phagocytized bacteria, might account for the recovery from the lungs of so many organisms. The facts, however, that collargol, India ink and other colloidal suspensions are found in the lungs in mononuclear and giant cells

and that in these experiments the bacteria were observed chiefly in the same types of cells, would seem to indicate that the mononuclear cells, rather than mobilized polymorphonuclear leucocytes, are responsible for the phagocytic activity of the lungs, at least in the rabbit. In the past many writers have claimed that the capillary endothelium in the lungs possessed great phagocytic powers, but few, at present, hold to this view. In the experiments described in this paper phagocytized bacteria were never observed in the lining endothelium of the blood vessels.

The presence of the mononuclear and giant cells in the various organs raises the question as to their origin. There is no evidence that the monocytes in the lungs are derived from the circulation or from the capillary endothelium. If they arose from cells normally present in the intima, such as connective tissue cells or histiocytes, one would expect to find numerous mitoses in the lesions, which was not the case. It is certain that the subendothelial lesions in the lung are preceded by perivascular collections of cells which have the staining properties of lymphocytes and that groups of similar cells constitute the initial lesions in the sinusoids of the liver. Furthermore, the earliest subendothelial pulmonary lesions are composed of cells of this type. If it is true that the monocytes are derived from these cells, this fact would confirm the contention that monocytes may originate from lymphoid cells, an idea brought forth by Maximow [42] and, more recently, supported by Bloom.[43] This would imply that the cells invade the relatively thin adventitial coats of the veins or migrate along the venules between the endothelium and muscular coats, starting at points in the capillary bed where the latter are negligible. The first possibility was never substantiated by the observation of actual invasion of the adventitia by perivascular cells, whereas the peculiar bulging up of the endothelium in large veins at the point of entrance of smaller ones certainly suggests the second. From a study of the lungs of rabbits injected with India ink or collargol it would appear that these substances are removed from the circulation and phagocytized by cells intimately connected with the capillary network and that the pigments are transported by these cells to different portions of the pulmonary interstitial tissue. Lucid explanations of the origin of the monocytes and the mechanism of formation of the subendothelial lesions must, for the present, be foregone.

The tissues of guinea pigs injected intravenously with non-hemolytic (gamma type) streptococci in doses comparable to those given rabbits failed to show any of the characteristic lesions in the lungs, liver and spleen. There was apparently an increase in the perivascular collections of lymphoid cells in the lungs, but monocytes and giant cells were never found in any of the organs. This would seem to suggest that the method of disposal in the guinea pig is different from that of the rabbit or that the bacteria in the doses given failed to stimulate any proliferative monocytic activity.

In conclusion one may say that the rabbit reacts in a very definite way to the intravenous injection of colloidal substances and of large amounts of certain kinds of bacteria. This foreign material is removed from the circulation chiefly in the sinusoids of the liver and spleen and in, or about, the capillary network of the lungs. This process of phagocytosis stimulates the production of cells which have the staining reactions of lymphoid cells and which apparently are eventually converted into monocytes and giant cells, and result in lesions which closely simulate those of tuberculosis. Such changes are most pronounced in the lungs, liver and spleen, but giant cells of the Langhans' type can be found in the bone marrow, glomeruli of the kidney and cortex of the adrenal. If the injected material is relatively stable (India ink, collargol and *B. tuberculosis*), it is found in the monocytes and giant cells, but one cannot say whether this phagocytosis is primary or whether cells already containing phagocytized material are, in turn, taken up. Such organ changes are usually accompanied by an increase of the monocytes in the circulating blood. The type of material injected seems to determine the organ in which the lesions are most prominent. With colloidal substances the lesions in the liver and spleen are most marked, with gamma type streptococci those in the lungs and liver, with *B. coli* those in the liver and with *B. tuberculosis* those in the lungs. The lesions produced by bacteria usually disappear promptly when the injections are stopped or when the rabbits become "immune" due to repeated injections, leaving little or no evidence of damage, other than a mild degree of fibrosis. The changes in the lungs following the injection of *B. tuberculosis* are more severe in type, probably due to the liberation of toxic substances from the slowly disintegrating bacilli, and, if such animals had been observed a longer time after the last injection, some definite damage to the lung structure would undoubtedly have occurred.

SUMMARY

1. Following the intravenous injection into rabbits of relatively large doses of various dead bacteria, there is a marked reaction of the tissues which contain cells of the reticulo-endothelial system. This reaction consists in an increase of lymphoid cells which are eventually transformed into, or replaced by, monocytes and giant cells. Such lesions, ordinarily, are temporary and result in no permanent damage.

2. Identical lesions occur after the intravenous injection into rabbits of various colloidal substances.

3. Such changes represent the reaction of normal rabbits in the disposition of foreign materials in the blood stream and have nothing to do with reactions secondary to sensitization or immunization.

The authors gratefully acknowledge the help given by Dr. David Seegal during the first part of the studies and the excellent technical assistance of Miss Miriam McKay.

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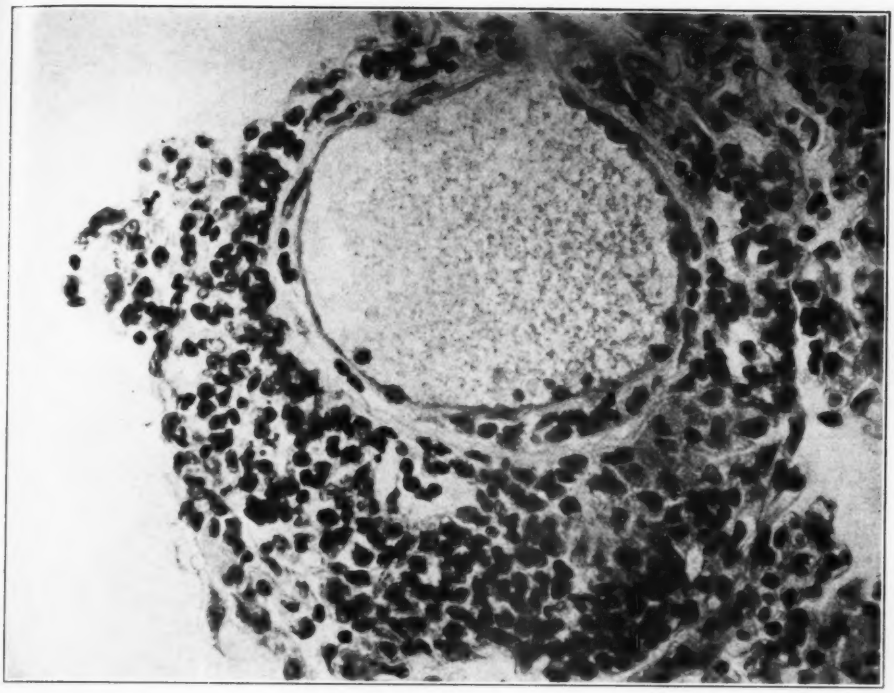
DESCRIPTION OF PLATES

PLATE 79

FIG. 1. Lung (No. 31). Perivascular collection of lymphoid cells. Very early subendothelial lesion. Eosin-methylene blue. × 500.

FIG. 2. Lung (No. 32). Two early subendothelial lesions, one composed chiefly of lymphoid cells and the other of monocytes. Eosin-methylene blue. × 250.



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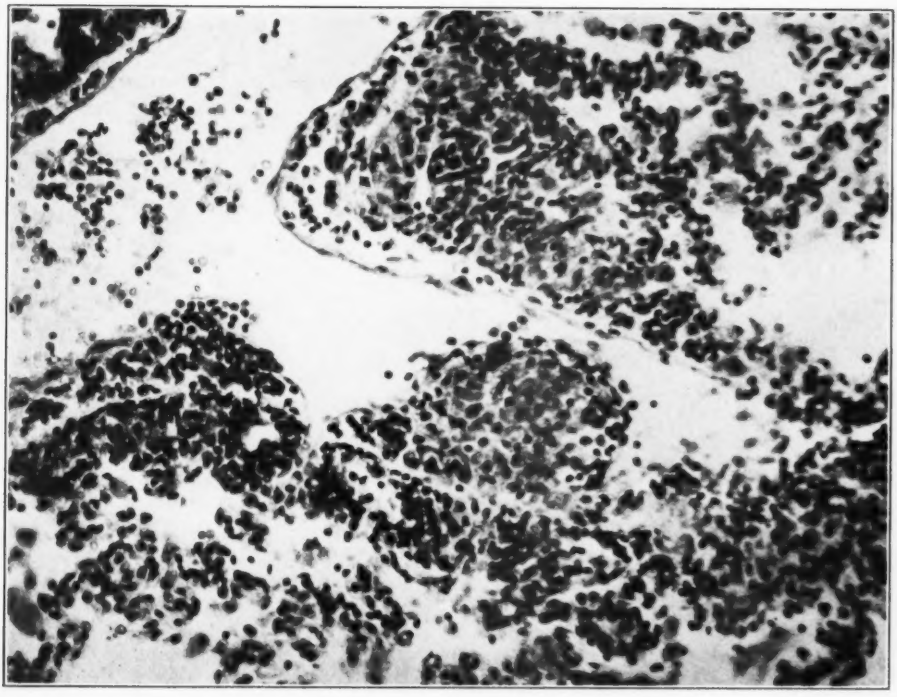


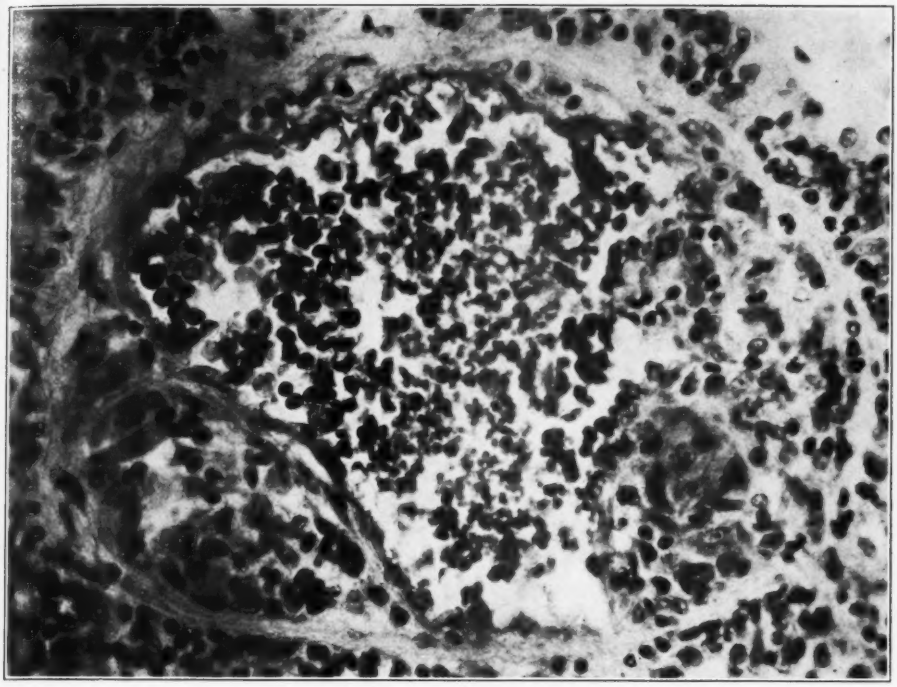
2

Nye and Parker Tissue Reactions Following Injection of Bacteria

PLATE 80

FIG. 3. Lung (No. 34). Subendothelial lesion, composed chiefly of monocytes. Eosin-methylene blue. × 500.

FIG. 4. Lung (No. 34). Subendothelial lesion with many monocytes, a few lymphoid cells and occasional giant cells. Eosin-methylene blue. × 200.



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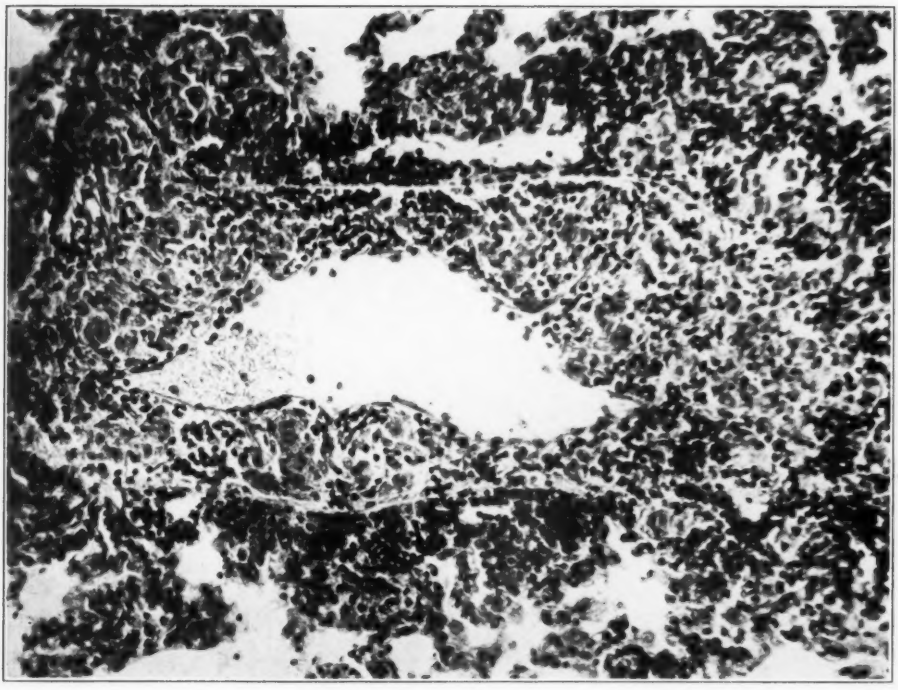


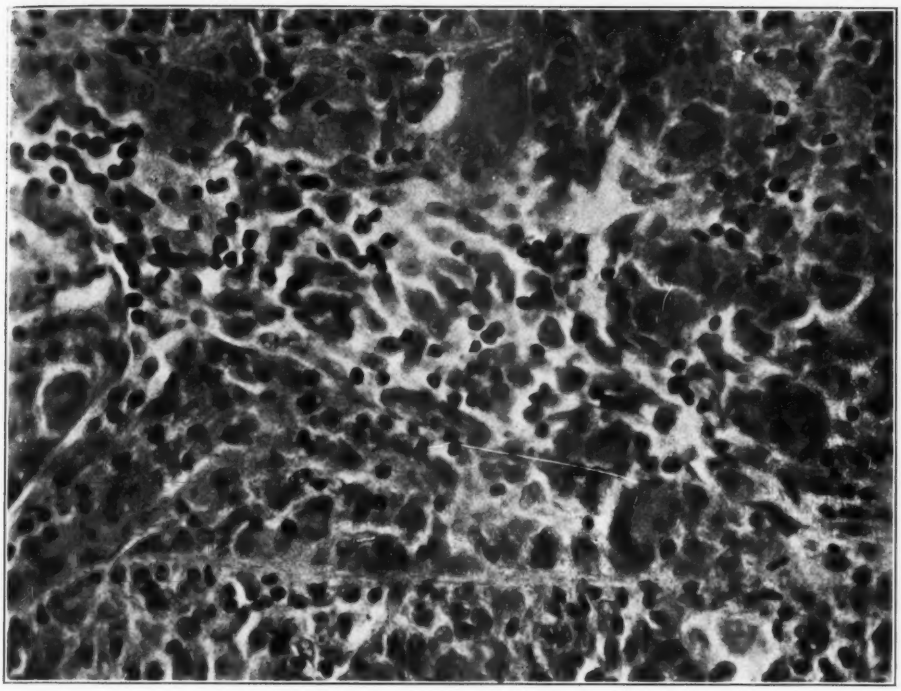
4

Nye and Parker Tissue Reactions Following Injection of Bacteria

PLATE 81

FIG. 5. Lung (No. 46). Almost complete obliteration of the lumen of the vein. Lesion consists chiefly of monocytes and giant cells. Eosin-methylene blue. × 500.

FIG. 6. Lung (No. 34). Subendothelial lesion with vessel wall emphasized by the staining of reticulum. Foot and Mènard's modification of Hortega's silver carbonate method. × 500.



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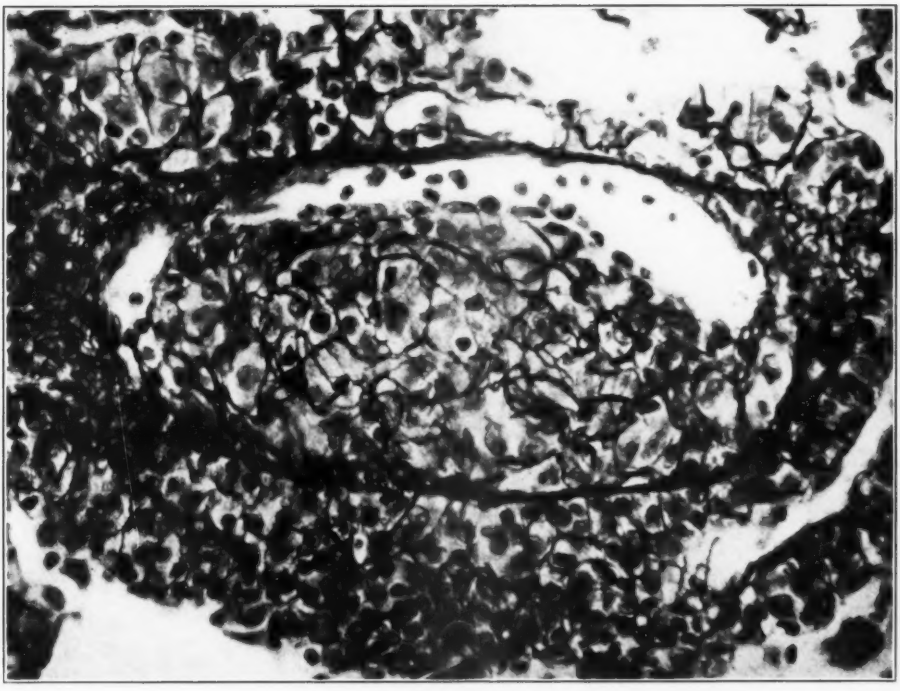


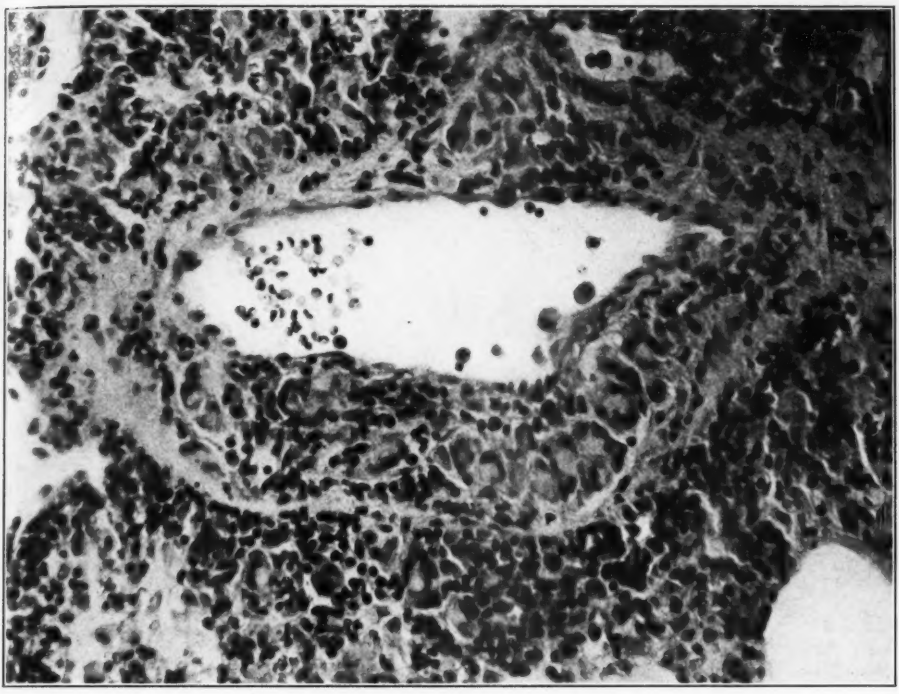
6

Nye and Parker Tissue Reactions Following Injection of Bacteria

PLATE 82

FIG. 7. Lung (No. 58). Subendothelial lesion. Eosin-methylene blue. × 300.

FIG. 8. Lung (No. 86). Early subendothelial lesion, chiefly lymphoid cells with a few monocytes. Eosin-methylene blue. × 500.



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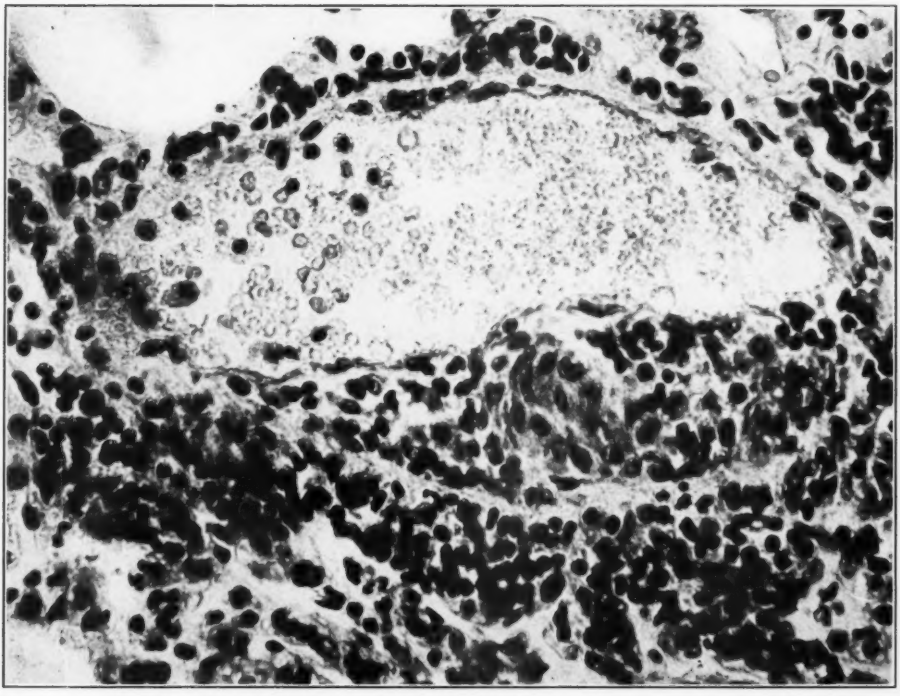


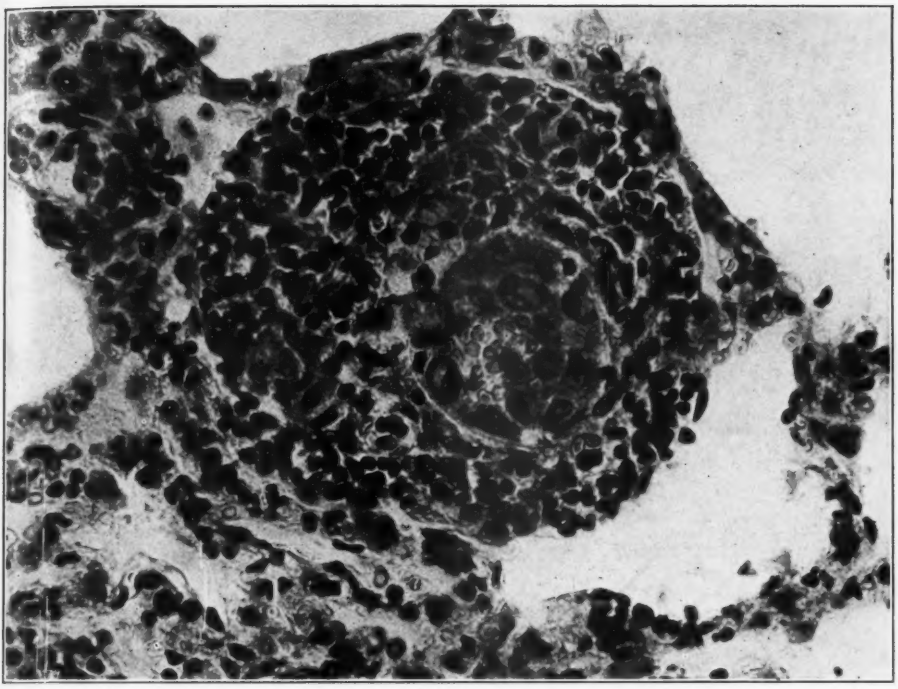
8

Nye and Parker Tissue Reactions Following Injection of Bacteria

PLATE 83

FIG. 9. Lung (No. 86). Nearly complete occlusion of the vein by monocytes with a marked perivascular accumulation of lymphoid cells. Eosin-methylene blue. × 500.

FIG. 10. Lung (No. 86). Complete occlusion of the vessel by a lesion simulating a true tubercle. Eosin-methylene blue. × 500.



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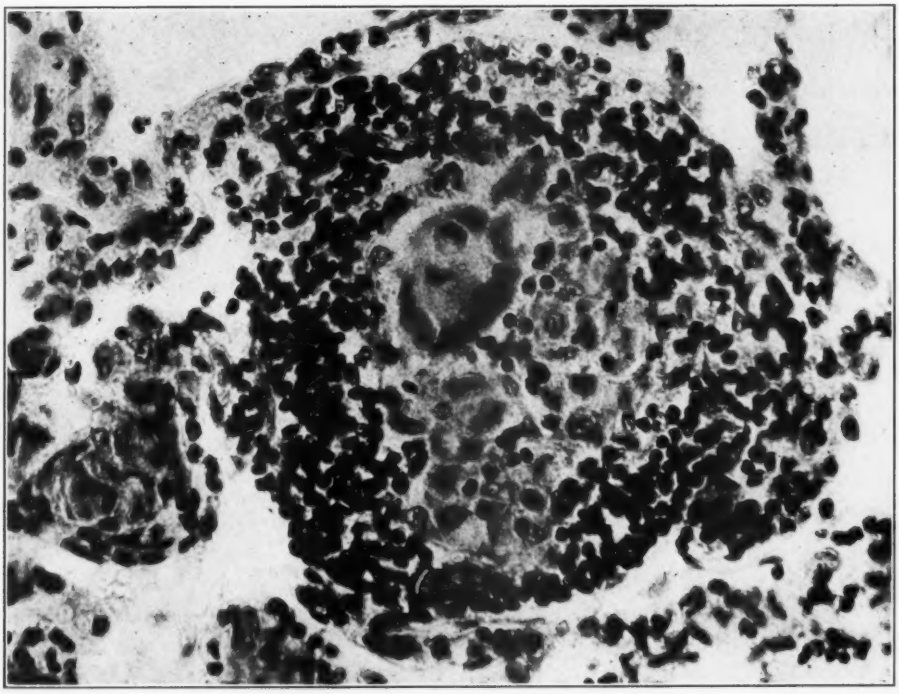


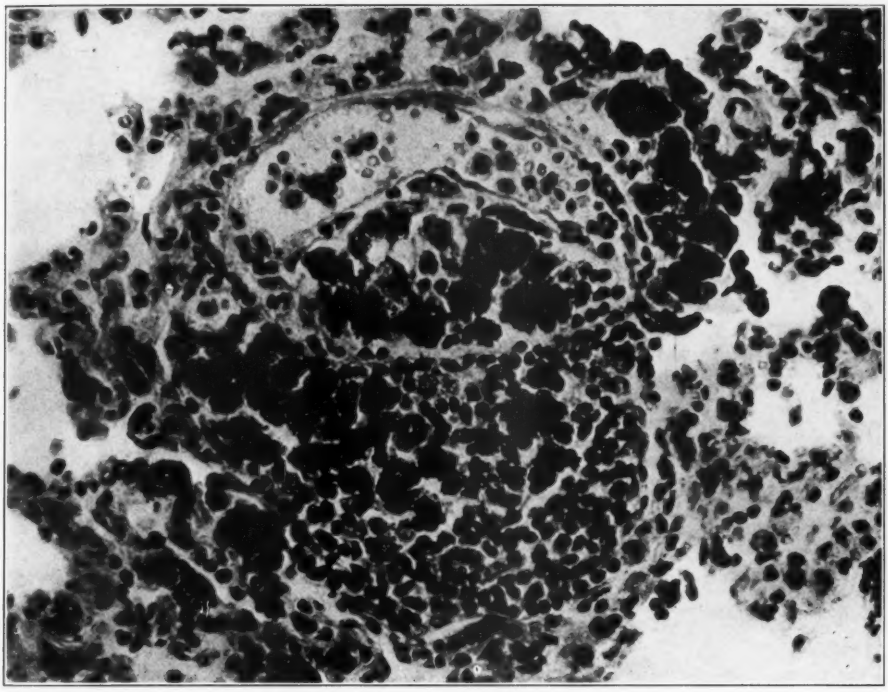
10

Nye and Parker Tissue Reactions Following Injection of Bacteria

PLATE 84

FIG. 11. Lung (12 intravenous injections of India ink at weekly intervals). Marked perivascular collection of lymphoid and pigmented cells, also subendothelial lesion composed chiefly of latter. Eosin-methylene blue. × 250.

FIG. 12. Lung (No. 38). Complete obliteration of the smaller veins with an apparent subendothelial extension of the process up into the large vein. Eosin-methylene blue. × 100.



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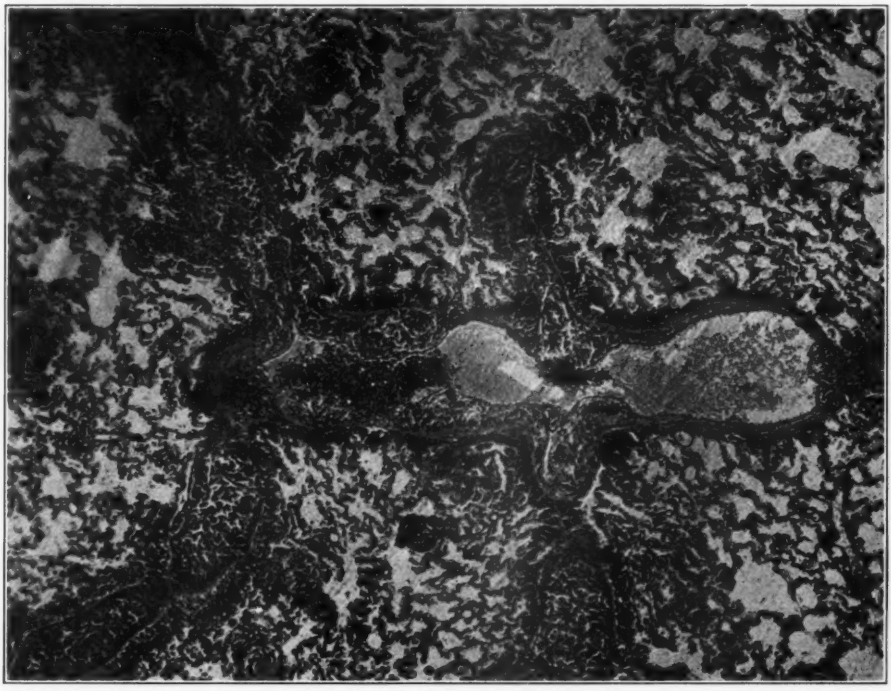

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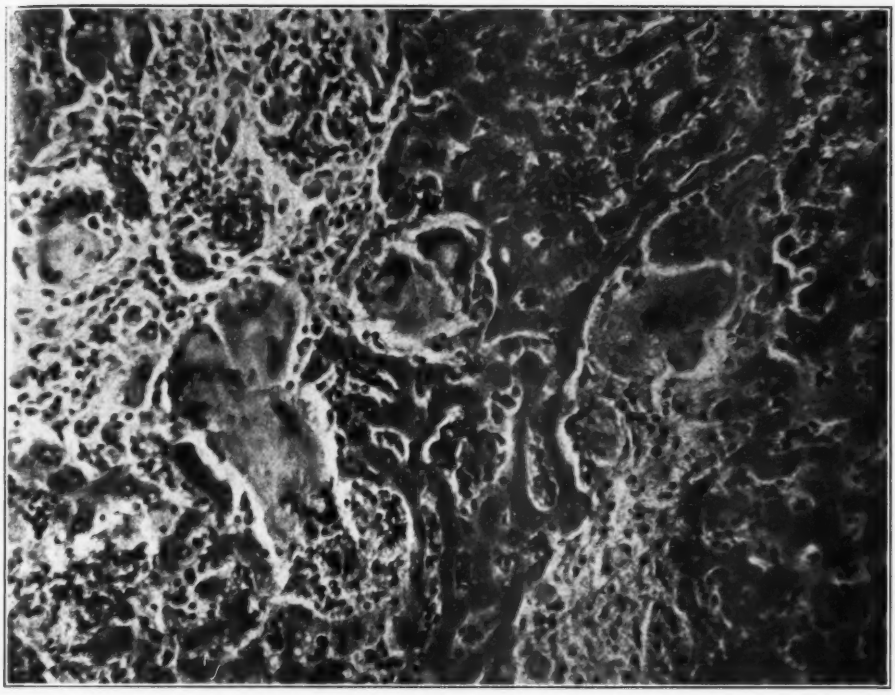
Nye and Parker Tissue Reactions Following Injection of Bacteria

PLATE 85

FIG. 13. Liver (No. 23). Small groups of lymphoid cells in the sinusoids, together with monocytes and giant cells. Eosin-methylene blue. × 250.

FIG. 14. Liver (No. 23). Monocytic "rosette" in a giant cell. Foot and Mènard's modification of Hortega's silver carbonate method. × 2000.



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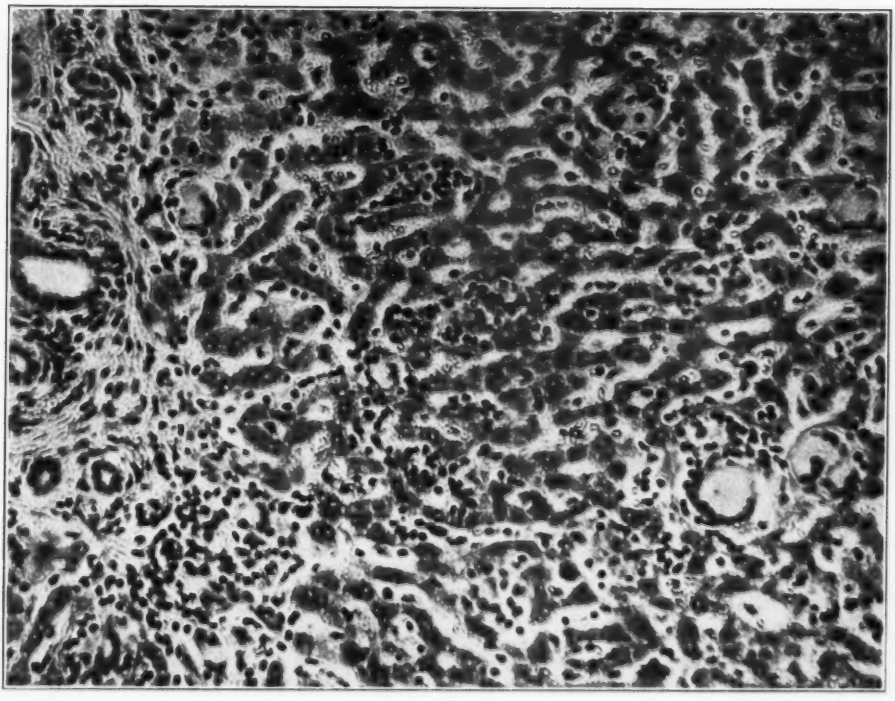

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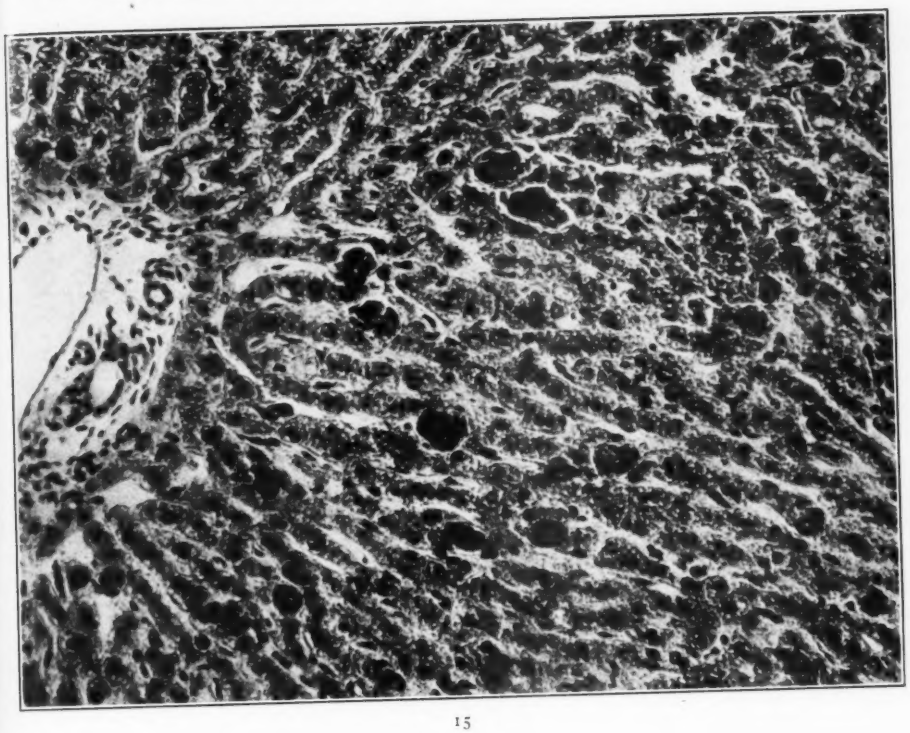
Nye and Parker Tissue Reactions Following Injection of Bacteria

PLATE 86

FIG. 15. Liver (No. 58). Lymphoid, monocytic and giant cells in the sinusoids. Eosin-methylene blue. × 250.

FIG. 16. Liver (7 daily intravenous injections of collargol). Pigmented mononuclear and giant cells in the sinusoids. Hematoxylin-eosin. × 250.



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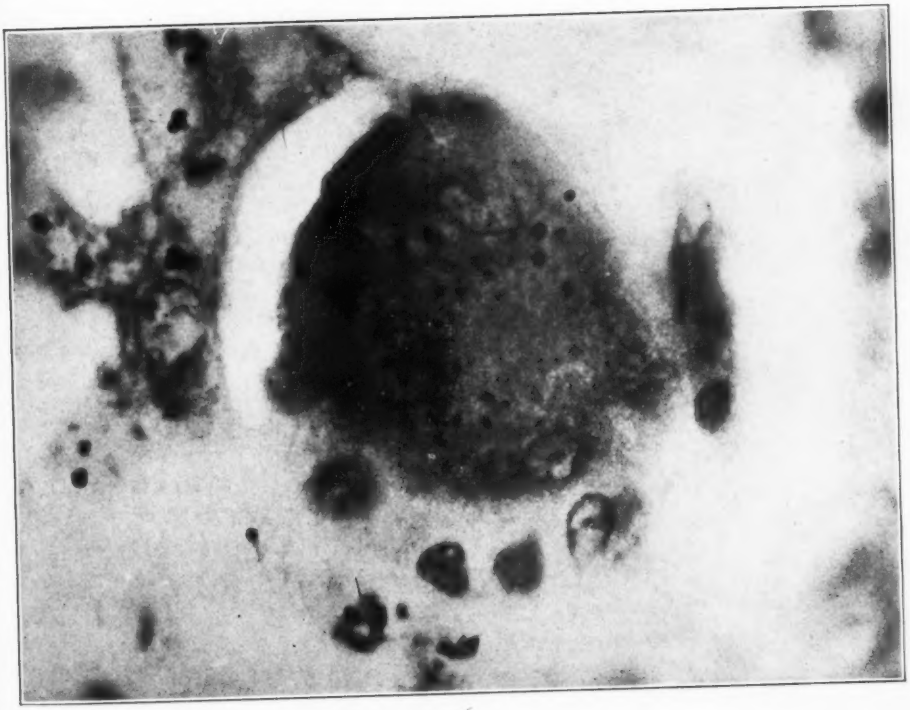

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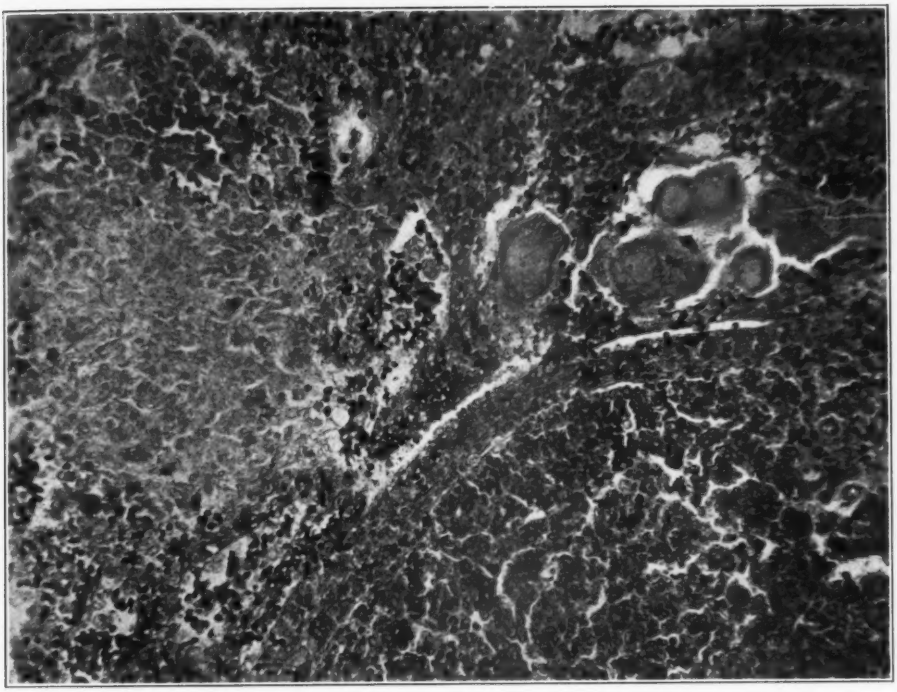
Nye and Parker Tissue Reactions Following Injection of Bacteria

PLATE 87

FIG. 17. Spleen (No. 34). Two foci, the one composed of monocytes, the other of giant cells. Eosin-methylene blue. × 200.

FIG. 18. Kidney (No. 26). Giant cell in a glomerulus. Eosin-methylene blue. × 1000.



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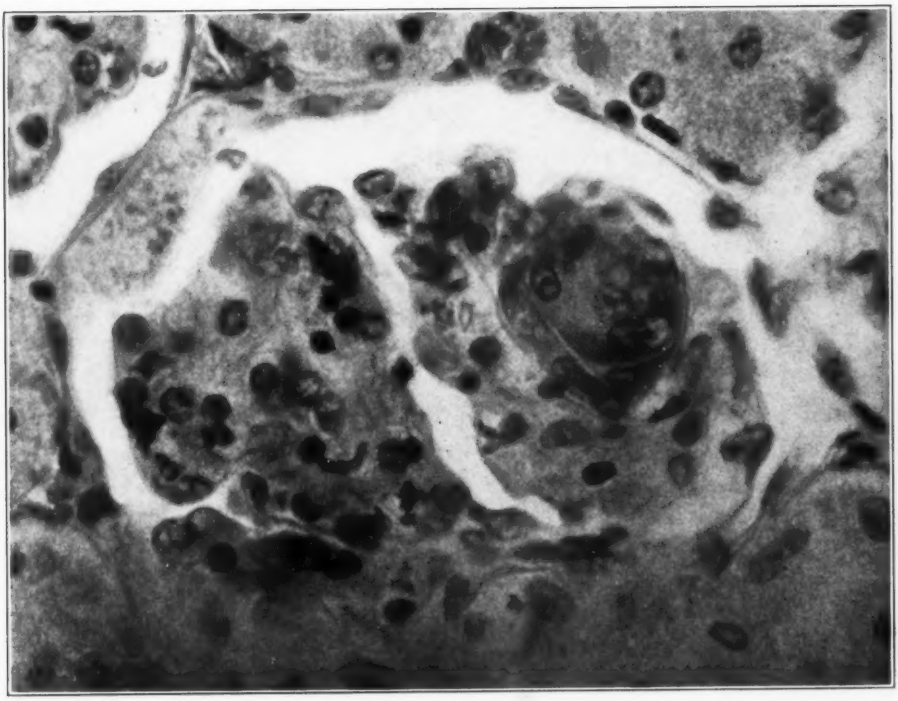

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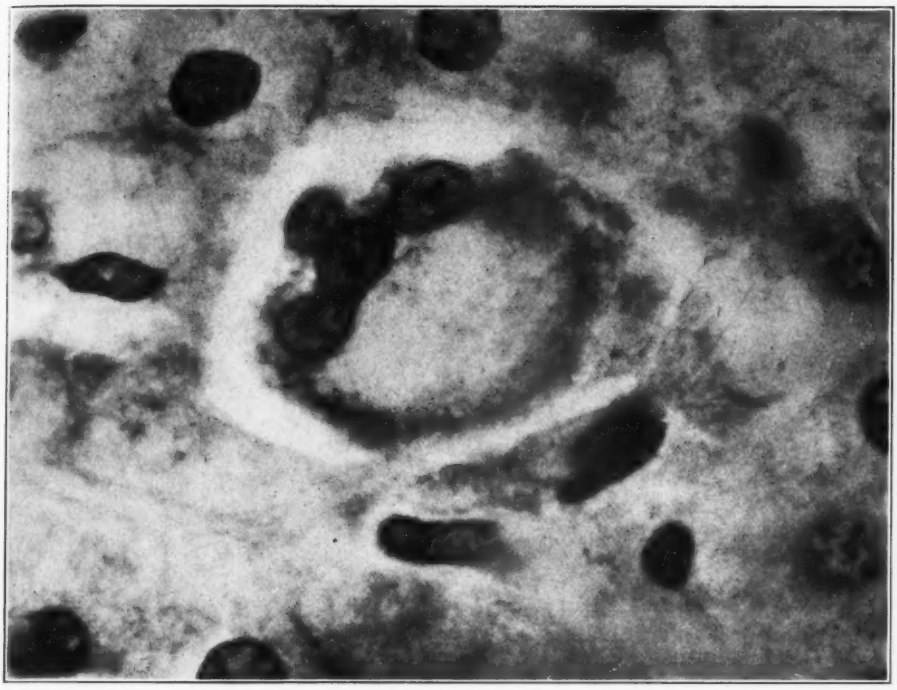
Nye and Parker Tissue Reactions Following Injection of Bacteria

PLATE 88

FIG. 19. Adrenal (No. 26). Giant cell in a sinusoid of the *zone fascsiculata*. Eosin-methylene blue. × 2000.

FIG. 20. Omentum (No. 39). Granulation tissue with two large giant cells. Eosin-methylene blue. × 500.



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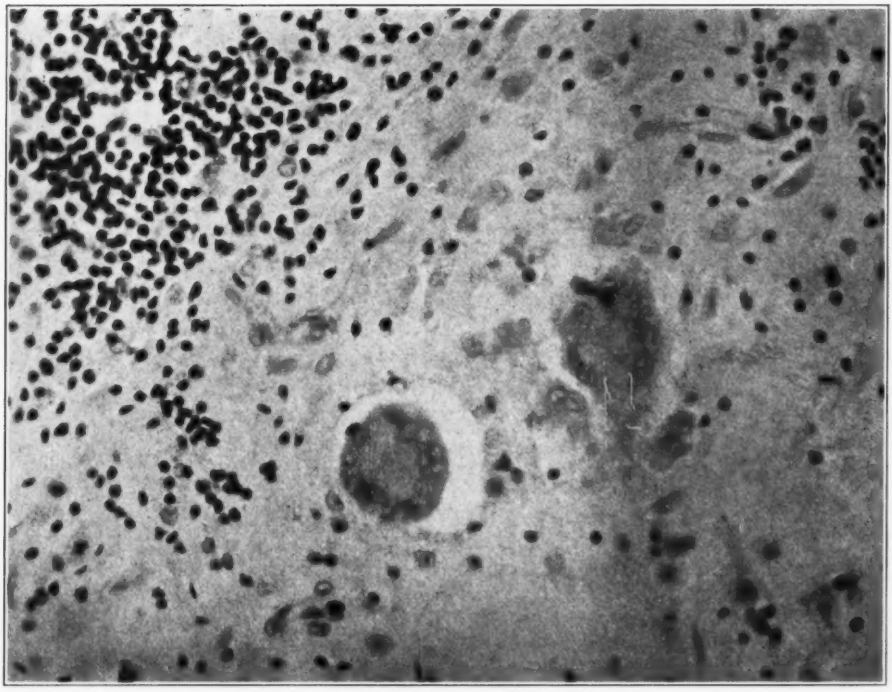

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A CLINICAL AND PATHOLOGICAL STUDY OF PERIARTERITIS NODOSA*

A Report of Five Cases, One Histologically Healed

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Introduction

Periarteritis nodosa was first described by Kussmaul and Maier sixty years ago as a disease characterized by the formation of multiple circumscribed nodular thickenings of the smaller arteries of various organs of the body. Since their classic description in 1866 about 150 cases have appeared in the literature. Several investigators have attempted to discover the etiology of this very interesting disease, and to determine the site of origin in the arterial wall. A specific microörganism has not yet been found, but we shall see that the evidence today is in favor of the specific infectious nature of the disease. A very acute case with death in a few days, published by Fishberg, has shown that the primary changes are usually in the inner media and not in the adventitia. The confusion which exists in the literature regarding the site of origin in the arterial wall is due to the fact that changes of different age occur in arteries of the same patient, and often in the same organ. Each acute exacerbation of the illness is accompanied by fresh changes somewhere in the body, and by new symptoms which vary with the localization of the arterial changes.

We shall attempt to summarize our present knowledge of periarteritis nodosa, and to divide the disease into four stages with a discussion of the pathology and clinical symptoms of each stage. This division is based upon a clinical and pathological study of five cases at the First Medical Clinic (Professor K. F. Wenckebach) and the Pathological Institute (Professor R. Maresch) of the University of Vienna. In addition, we had the opportunity of studying three more cases postmortem. One of our cases reveals the histologically healed end-stage involving every organ of the body except the central

*Received for publication March 3, 1930.

nervous system. The study of this case has made it possible to complete the pathological picture of the disease, and to describe a new clinical syndrome caused by histologically healed periarteritis nodosa.

Periarteritis nodosa is an inflammatory disease of the arterial system, probably caused by a filterable virus, and characterized by necrosis of the media with fibrinous exudation. This is soon followed by a marked cellular infiltration and granulation tissue formation in and about the arterial wall, together with varying degrees of intima proliferation. The chief secondary changes in the arteries are aneurysm formation, thrombosis and hemorrhage. Death is usually due to hemorrhage from rupture of an aneurysm, or to necrosis or insufficiency of vital organs resulting from thrombosis of the arteries. The veins are free from changes in almost all the cases studied.

At present we know of no predisposing causes of this disease. There seems to be no relation to occupation. However, the disease is four times as frequent in males as in females. It may occur at any age: the youngest patient reported was an infant of three months, the oldest 78 years. About 50 per cent of the cases are found between the ages of 20 and 40 years. The duration of the illness is usually a few weeks to six months, rarely longer than a year. Fishberg's case was ill only six days. Our case of histologically healed periarteritis nodosa lived four years after his one and only attack of acute illness.

Our knowledge of the early changes in the disease is now quite complete except for the question of involvement of the endothelium of the affected arteries. The endothelial changes are difficult to demonstrate, but we believe that they do occur. We know that the most marked changes develop in the media, and this fact has led to the question whether periarteritis nodosa is not related to the changes found in the arteries by Wiesel, von Wiesner, and others in various infectious diseases. They have found degenerative changes in the media which they believe are related to the development of arteriosclerosis in some arteries, such as the coronary. Recently Pappenheimer and VonGlahn have found similar changes in the arteries in rheumatic fever. Spiro has attempted to bring these changes in close relation to those found in periarteritis nodosa, and considers the latter no disease *sui generis*, but only a form of postinfectious mesarteritis. Gruber is also of the opinion that peri-

arteritis nodosa is a disease of toxi-infectious origin without a specific cause. We cannot share this view, and we believe from our study of five cases of this disease that we are dealing here with a specific infectious disease, the virus of which has an elective affinity for the arterial system and enters the arterial walls directly from the lumen and through the vasa vasorum in the larger arteries.

The view that syphilis is the cause can now be dropped entirely. To be sure, a luetic patient may also contract periarteritis nodosa, but even the cases with a positive Wassermann have shown changes characteristic of periarteritis nodosa and not of luetic arterial disease. Most cases have a negative Wassermann, spirochetes have never been found in the lesions, gummas are not found, giant cells very rarely occur, and the localization is different from that of syphilis. Also the occurrence of periarteritis nodosa in lower animals speaks against syphilis as a cause. The view of several early writers that it attacks individuals with a hereditary or acquired weakness of the arterial walls need no longer be considered.

The most promising work on the etiology of the disease is that of Harris and Friedrichs, and of von Haun, who have succeeded in producing quite similar changes in experimental animals. Furthermore, the finding of a similar disease in lower animals (calf, swine, dog, deer), where it at times occurs in epidemic form, also speaks for the specific infectious nature of the disease.

The organs most frequently attacked are the kidneys (80 per cent), heart (70 per cent), liver (65 per cent), gastro-intestinal tract(50 per cent), pancreas (25 per cent), mesenteric artery (30 per cent), muscles (30 per cent), and peripheral nerves (20 per cent). *The central nervous system is involved* in only 8 per cent of the cases. The disease may also remain confined to a single organ for some time.

We shall divide the disease into four stages according to the changes present in the arteries, and discuss the clinical and pathological findings in each. These are: (1) alterative-degenerative or beginning stage; (2) acute inflammatory stage; (3) granulation tissue stage, and (4) healed end-stage, or scar tissue stage.

First or Degenerative Stage

Pathology: The beginning stage is characterized by alterative-degenerative changes in the media, with edema and the appearance of a thready fibrinous exudate about the elastica interna. There is

a swelling of the muscle cells with separation by the exudate. *In the smaller arterioles*, those without vasa vasorum, the changes are chiefly in the innermost media, in the subendothelium. No doubt the causative agent enters from the lumen producing endothelial changes as well. A part or the entire circumference of the vessel wall undergoes a coagulation necrosis with a hyaline-like appearance of the inner media. The endothelium may also be affected so that the cells become desquamated or disappear over the affected area. The subintimal changes, the edema and fibrinous exudation may elevate the endothelium causing it to bulge into the lumen or even reducing it to a narrow slit. The fibrin network may extend through the endothelium into the vessel wall. *In the larger arteries*, those with vasa vasorum, the changes appear more often in the outer media, lying near the elastica externa. Here the hyaline areas of necrosis appear and seem to go out from the vasa vasorum or from smaller branches of the vessel. Of course, when the necrotic changes become more extensive they may also reach the endothelium in these vessels. Usually a portion of the vessel circumference presents in the media an area of necrosis with edema and fibrinous exudation. Serial sections often show this region to extend longitudinally in the vessel wall. The areas form an ellipse with the long diameter axially arranged, less often transversely. The entire thickness of the media may become hyalinized. Leukocytes begin to wander into the necrotic area. They are chiefly polymorphonuclear neutrophiles, though eosinophiles may also be present in considerable numbers. In this stage the adventitia is often unaffected.

In the early stage changes may also be present in the large arteries and even in the aorta. We have found in one case fragmentation of the elastica interna, with vacuolization and round cell infiltration about the elastica interna in large arteries. That endothelial changes occur in periarteritis nodosa is shown by findings in the brain in one of my cases. Here we observed circumscribed hemorrhages forming a ring about a number of small arterioles, without any demonstrable change in the vessel wall. We must assume, therefore, an increased permeability of the endothelium with hemorrhage by diapedesis into the surrounding lymph space.

Clinical Symptoms: In this beginning stage the disease is often latent, especially when the changes are limited to a small area, hence the difficulty of determining the exact age of changes seen post-

mortem. At this stage the diagnosis is not possible, the changes are still microscopic. There may be no symptoms whatever; or only a rise of temperature. We hardly need state that we cannot sharply separate these stages from one another. The earliest case in the literature, that of Fishberg, with clinical symptoms of only six days duration, died of renal insufficiency due to extensive infarction of both kidneys. Fever, hematuria, icterus, myocardial insufficiency or pains in the extremities often mark the onset of the disease.

Second or Acute Inflammatory Stage

Pathology: This is the exudative inflammatory stage. A great infiltration of the media and adventitia with polymorphonuclear neutrophiles, sometimes also many eosinophiles, lymphocytes and plasma cells rapidly takes place. The fibrinous exudate extends to the intima and outward in the adventitia. There is destruction of the inner media and of the elastica interna which becomes stretched and fragmented. The process may also extend through the adventitia and spread by way of the perivascular lymphatics. The perivascular connective tissue becomes edematous and leukocytes appear in large numbers. There may be destruction of the entire vessel wall over a part or all of its circumference. The muscle cells of the media become separated from one another and then undergo complete necrosis, the elastica becomes fragmented, survives longer than the muscle cells, but soon also disappears. When the exudate reaches the intima the fibrin threads may penetrate into the lumen and leukocytes wander from the lumen into the subendothelial tissue. A marked subendothelial connective tissue proliferation takes place, a reactive intima proliferation. At the height of this stage secondary thrombosis of the lumen with infarction of the various organs is common. Toward the end of this stage aneurysm formation or rupture of the vessel wall with hemorrhage into the adventitia or surrounding tissue occurs. There is no suppuration, and pyogenic bacteria are not found. In cases without aneurysm or nodule formation the changes may be overlooked without microscopic examination.

Clinical Symptoms: This is the stage with high fever, chills, a polymorphonuclear leukocytosis (sometimes also an eosinophilia) and all the symptoms of a severe infection. The fever may be con-

tinuous or intermittent. After a time a secondary anemia develops. The symptoms vary with the location of the process and the nature of the secondary changes in the vessels. Renal, cardiac, peripheral nerve, and gastro-intestinal symptoms are the most common. Icterus may also develop due to involvement of the liver. The spleen is often, though not always, enlarged. There are frequently somewhat enlarged, hard lymph glands. The pulse is as a rule regular and accelerated even when the fever has disappeared.

Death is frequent in this stage, due to rupture of an aneurysm with fatal hemorrhage. This may occur in any of the affected organs. Or, renal insufficiency due to extensive infarction may cause death. Another common cause is cardiac failure due to extensive coronary involvement with thrombosis or intima proliferation.

The finding of subcutaneous nodules, which on histological examination show the characteristic arterial changes, offers a means of making a sure diagnosis *in vivo*.

Third or Granulation Tissue Stage

Pathology: This is the reparative or granulation tissue stage. There is a marked proliferation of fibroblasts from the adventitia into the inflammatory zone, accompanied by a reduction in the polymorphonuclear leukocytes with an increase in the lymphocytes and plasma cells. Sometimes eosinophiles appear in considerable number. The fibrin network and hyalinized necrotic media are gradually replaced by the cellular granulation tissue rich in fibroblasts and newly formed blood capillaries. This granulation tissue not only replaces the destroyed media, but also extends outward through the adventitia, and longitudinally as well as circularly beyond the area of destruction. It may also penetrate the subendothelial tissue through the defects in the elastica interna. It may even pass through the entire wall and invade thrombi formed in the lumen.

In addition to the granulation tissue formation there is usually a very marked reactive intima proliferation with a partial or total occlusion of the lumen. This intimal thickening consisting of a loose fibroblastic connective tissue usually extends beyond the area of destruction of the media. It is often circular, but may also extend longitudinally on only one side of the vessel. The proliferation is

usually thickest at the site of the lesion with the result that the narrowed lumen often lies eccentrically on the less affected or unaffected side. The spread of the intima proliferation beyond the area of media destruction explains why we so often find in transverse sections a marked intimal thickening without changes in the vessel wall. We have convinced ourselves by a study of many serial sections from different organs that these areas lead to vessel wall changes usually at the place where the intimal proliferation is most marked. In other words, the intimal thickening in periarteritis nodosa is usually the consequence of vessel wall changes in the media.

In this stage of granulation tissue formation, aneurysms or rupture of the wall may occur or there may be only a thickening of the wall from intimal proliferation, or granulation tissue formation outside the adventitia. Cases without nodule formation on the vessels may be overlooked unless examined microscopically.

Clinical Symptoms: In this stage marked anemia, emaciation and marasmus usually develop if the involvement is widespread. If confined to a non-vital organ or tissue, healing may occur, clinical as well as histological. The fever and leukocytosis usually drop or may entirely disappear. However, acute exacerbations are the rule, with the development of fresh foci. The symptoms in this stage are due to vascular occlusion of the kidneys, heart, gastro-intestinal tract, peripheral nerves, muscles, and glands of internal secretion. They are hypertension, nephritis, renal insufficiency, cardiac failure, intense pains in the abdomen, icterus, ulcerations or gangrene of the bowel, peripheral neuritis, muscular atrophy, Addisonoid symptoms, etc. Or a sudden collapse due to internal hemorrhage may occur (kidneys, liver, gall bladder, gastro-intestinal tract, pancreas, lungs, brain, etc.). The diagnosis of rupture of an artery can then be made. Such hemorrhage is more common in the second stage of the disease.

Fourth or Healed Granulation Tissue Stage

Pathology: This is the histologically healed end-stage or scar tissue stage, as illustrated by the case of periarteritis obsoleta nodosa of four years duration, which we shall describe. The destroyed arterial wall, often only the inner media including the elastica interna, is replaced by an indifferent fibrous scar tissue poor in nuclei. The lumen is greatly reduced in size or totally obliterated. Often only

narrow channels run axially through the greatly thickened vessels. Nodular thickenings of the outer wall occur when the process involves the outer media and adventitia. Then a marked periarterial fibrosis takes place as a result of the histological healing of the granulation tissue in the adventitia and surrounding connective tissue.

Three processes here are active, separately or very often combined: (1) a marked subendothelial connective tissue proliferation which may be accompanied by the new formation of elastic fibrils and often extends beyond the area of media destruction; (2) thrombosis of the injured vessels with complete organization with or without recanalization, and (3) healed granulation tissue scar formation in and about the injured vessel wall. These three processes can often, but not always, be distinguished from one another.

Only cases of severe periarteritis nodosa with a tendency toward thrombosis and intimal proliferation will reach this advanced stage. The cases with aneurysm formation usually lead to fatal hemorrhage. Also, early complete occlusion of the blood supply to vital organs, whether by thrombosis or intimal proliferation or both, will result in early death. It is, therefore, possible for a generalized periarteritis nodosa to become histologically healed only when the blood supply is not reduced below the minimum necessary for the maintenance of function of the vital organs. Case 5 will demonstrate this fact very well. No disease better illustrates the great factor of safety in the blood supply to vital organs than does periarteritis nodosa.

The intimal proliferation is a reactive local proliferation in response to the injury of the inner media. It is characterized by the absence of blood capillaries and hemosiderin deposits such as are seen in organized granulation tissue or thrombosis. The fibers are arranged more or less concentrically in cross-sections of the artery. The elastica interna presents all stages of degeneration and necrosis. A new formation of elastic fibrils with marked thickening of the intima occurs in arteries where the changes in the media are less severe and hence the elastica has not been destroyed.

The healed granulation tissue which grows into the adventitia and media is characterized by a richness in fibroblasts, the presence of numerous newly formed blood capillaries, and fine deposits of hemosiderin. As this tissue grows older it becomes more and more hyaline and fibrous, the capillaries are compressed and obliterated,

and the hemosiderin slowly disappears. The healed scar tissue remains as evidence of the severity and extent of the earlier acute inflammatory process. The perivascular mantles of scar tissue, which we have found surrounding the arteries with extensive destruction, we consider to be characteristic for this healed end-stage. These arteries lie embedded in thick sheaths of scar tissue radiating through the affected organ, producing the appearance of an interstitial scar tissue formation. Where the arterial destruction is greatest there the periarterial healed granulation tissue is the thickest.

The final organ changes in this stage are contracted kidney, contracted scarred liver (hepar lobatum), myomalacia scars, adrenal atrophy, necrosis or ulceration in the gastro-intestinal tract, encephalomalacia, muscle atrophy, and peripheral nerve degeneration. In other words, we may have healed infarcts or atrophy in any of the organs with characteristic arterial changes. The vessel changes may be microscopic or macroscopic. With severe changes there are often nodules on the arteries produced by healed aneurysms or periarterial scar tissue formation. Elastic tissue stains of the arteries, and serial sections should be made in all suspected cases.

Clinical Symptoms: There is an absence of fever in this end-stage when, as in my case, all the lesions in the body are histologically healed. The pulse remains accelerated, but is usually regular. The leukocyte count is normal. The symptoms are due to a progressive reduction of the blood supply in the various organs, and may be as variable as in the earlier stages, depending upon the localization of the vascular process. We can expect as most common: renal insufficiency, cardiac failure (without pulmonary or other demonstrable cause) which is resistant to the action of digitalis, degenerative polyneuritis, marasmus, muscular atrophy, abdominal cramps, hepar lobatum, gastro-intestinal ulceration, encephalomalacia, adrenal insufficiency or even polyglandular insufficiency.

The difficulty in the diagnosis of the disease in the earlier stages seems to be even greater in this histologically healed end-stage. The history of a previous severe febrile attack, the symptoms of renal involvement, polyneuritis and polymyositis, and abdominal pains, that most common tetrad of symptoms in periarteritis nodosa, might enable one to diagnose the healed end-stage. The finding of nodules in the skin with the characteristic histological changes would render possible the diagnosis.

Case Reports

Case 1. *Clinical History:* The patient, Franciska H., a dressmaker 46 years of age had the following history: Both parents died of old age. The patient has four sisters, one has heart disease, another gastric ulcer. The patient was always well until three years before her present illness when she had pneumonia. A year ago she had a "rheumatism" confined to the left shoulder joint.

The present illness began six weeks before entrance into the hospital with intense pain in the leg muscles so that the patient couldn't walk. Soon similar pains developed in the arms and hands. She stated that she had no fever at this time. About fourteen days ago the patient suddenly developed high fever to 40° C, with chills and slight sore throat. The angina disappeared after a few days, but not the fever.

Examination showed a medium-sized woman with poor musculature and very little subcutaneous fat. The cranial nerves were all free from disturbance. In the chest there was a slight dullness over both apices, but the lung borders were normal. The heart was not enlarged. There were no murmurs. The liver was slightly enlarged on percussion. The spleen was not palpable.

The pulse rate was 100–120 but regular.

There was marked tenderness on pressure over the sciatic nerve, also the peripheral nerves of the upper extremities. The patellar reflex was present, the Achilles' reduced. There was no Babinski, no clonus.

The white blood count was 12,400. The Wassermann was negative. Blood cultures taken at the height of the fever were negative. Repeated examination of the sputum failed to reveal tubercle bacilli. The patient's septic temperature continued unchanged in spite of the use of aspirin, electrocollargol, Pregl's solution, etc. The heart action remained good and no signs of endocarditis could be found.

Twelve days after entrance, on October 24, the pains in the arms increased. There were sensory disturbances in the radial nerve distribution of the right hand. The reflexes in the left arm were increased. On November 3, the condition of wrist-drop developed on both sides. The radialis no longer reacted to galvanization or faradization.

On November 5, the findings in the arms were as follows:

Right Arm: The patient could carry out all movements in the shoulder joint and elbow, but only slowly and with effort. The movement of the wrist and finger joints was practically impossible. The radialis showed no reaction to faradic or galvanic current. The ulnaris reacted slightly to faradization.

Left Arm: There was slight improvement, the radialis and ulnaris reacting to faradic with slow contraction of the muscles.

Right Leg and Left Leg: The peronealis reacted but the tibialis did n't.

On November 12, the reflexes were absent in the lower extremities. The reaction of the nerves of the arms varied, one day they reacted slightly, the next day not at all.

The patient became weaker every day and drugs were ineffective. On November 19, the patient collapsed and received camphor and caffeine. On November 28, the patient developed severe pain in the abdomen; the large intestine could be palpated and was strongly contracted. There was a slight diarrhea with bloody stool. On November 29, the patient became comatose, and developed a right-sided facial paralysis. Speaking was difficult, also swallowing. The patient died on November 30, in deep coma.

The clinical diagnosis was infiltration of the right upper lobe with abscess formation, polyneuritis, metastatic process in cerebro, enteritis.

The autopsy, performed by Dr. Feller, revealed an extensive periarteritis nodosa involving almost all the organs of the body, including the central nervous system as well as the peripheral nerves. Most marked are the changes in the gastro-intestinal tract. In the stomach there are infiltrations on the arteries up to the size of a pea along the greater and lesser curvatures. The nodules in large numbers produce protuberances of the mucosa so that the inner surface of the stomach appears nodular. Especially numerous are the nodules about the small arteries at the mesenteric attachment of the small and large intestine. Also in the intestines the nodules often cause protrusion of the mucosa into the lumen. In places the intestinal wall appears to be undergoing necrosis. In the large intestine (cecum, ascending and transverse colon) are a number of bleeding ulcers of the mucosa, some covered with a necrotic membrane. These vary in size up to 2 cm. In the ileum longer stretches of the wall are necrotic. There is a circumscribed fibrinous peritonitis over these areas.

Miliary to pea-sized nodules are found on the peripheral arteries. In the extremities along the muscle and nerve branches are numerous pinhead to millet-seed-sized nodules. Many nodules are seen in the liver along the branches of the hepatic artery, in the kidneys and in the pancreas. There are many nodules and areas of thickening on the coronary arteries.

A large fresh cerebral apoplexy exists in the region of the left basal ganglia and reaching almost to the cortex, with perforation into the left lateral ventricle. A confluent lobular pneumonia is present in the right lung, with fibrinous pleuritis.

Histological studies showed acute and chronic changes in the arteries of every organ in the body. I wish to call attention to the presence of multiple small periarteriolar hemorrhages in the brain, in some places without demonstrable arteriolar change other than a slight swelling or edema of the wall. The very extensive degeneration of the peripheral nerves, always accompanied by severe arterial changes with obliteration of the lumen in many places, is of special interest in this case. Also the marked involvement of the gastro-intestinal tract with numerous nodules in the submucosa commands our attention.

Summarizing our observations in this case we find:

A woman of 46 years developed a severe polyneuritis with pain, marked weakness, and atrophy of the muscles of the extremities, loss of reflexes and wrist-drop. This was accompanied by a persistent high septic temperature, a rapid regular pulse. Then came a severe collapse, followed by intense abdominal pain and bloody diarrhea. Death was due to cerebral apoplexy with rupture into the lateral ventricle. The autopsy revealed a generalized periarteritis nodosa. This variety of symptoms: weakness, septic temperature, polyneuritis, abdominal pain followed by melena, and cerebral hemorrhage, could not be "brought under one hat" by the clinician. We want to emphasize this very fact as characteristic of most cases of periarteritis nodosa. When we find such a variety of symptoms, referable to various organ systems, we should think of a common vascular cause such as periarteritis nodosa.

CASE 2. *Clinical History:* Joseph H., aged 55 years, had always been well. The family history revealed nothing of importance. Except for a severe burn sustained by the patient fifteen years ago he had always been well. The patient entered the hospital on October 20, 1925.

The present illness began three weeks ago with intense pain in the right calf of the leg. The pain then spread to the knee region and the toes. He had the feeling that the leg was swollen. The pain was more severe on walking than on lying in bed. There was a sensation of numbness in the leg, with paresthesia at times. A week later the same symptoms developed in the left leg, but not so severe as in the right. Still he could continue his work until October 16, four days before entrance into the hospital. On October 17, the pains became so intense that the patient was forced to bed. At this time similar symptoms developed in the left forearm and hand. The left extremity was weaker and anesthetic. The patient also had been vomiting two or three times a week in the morning for five weeks.

The patient drank five to six beers and about one quarter of a liter of wine daily. He had been a heavier drinker. Venereal disease was denied. The patient smoked, but not to excess.

We shall not give the complete physical findings, but only those facts of interest in connection with the disease.

The patient was a medium-sized well developed man in good nutritional condition. There was no edema, icterus or cyanosis. The head was entirely normal, the pupils reacted normally to light and accommodation. The thyroid was not enlarged, there were no abnormal glands palpable.

The heart and lungs were normal, except for a few râles at the base of the right lung posteriorly. The pulse was 100, regular. The temperature of the patient was remittent, rising afternoons to as high as 39° C. The liver and spleen were not enlarged. There was no abnormal resistance in the abdomen. The blood pressure was 145 to 160 mm. systolic. The Wassermann test of the blood was negative.

The movement of the left arm and both legs was considerably reduced, as also

the strength in these extremities. The patient dragged his foot somewhat on walking. The patellar and Achilles' reflexes were somewhat reduced.

The patient perspired considerably. He developed paresthesias in the right hand on October 24. On October 27, the pain in the calves disappeared, and the paresthesia was less marked. The patellar and Achilles' reflexes were gone. There was a slight edema over the internal and external malleoli. The muscle sense of the fingers and wrist was greatly disturbed. The right hand developed a wrist-drop with hyperesthesia in the radialis region. There was a marked atrophy of the interossei muscles.

On November 3, the edema about the ankles was still present. The temperature rose daily to 38 or 39° C. Both arms could be moved only with difficulty. The urine showed a trace of albumin, but no renal elements.

On November 10, the pain in the calves was still present. The heart dullness was enlarged, the pulse 104. On the 15th, the sensory disturbances of the ends of the lower extremities were somewhat reduced. The patient could lift his right foot a little better. On November 20, the movement of the wrists and finger joints was somewhat better.

On December 2, the patient developed severe dyspnea, with numerous bronchial râles over the entire lung. He had a tachycardia. The liver was enlarged, hard and painful to pressure. There was no ascites. On December 7, dullness was found over the right base posteriorly. The heart apex lay in the 6th interspace in the anterior axillary line. The edema of the lower extremities and sacral region was increased.

The patient died on December 15, after an illness of about ten weeks. The clinical diagnosis of Professor H. Schlesinger was: polyneuritis alcoholica, myodegeneratio cordis with marked decompensation, pneumonia.

The following laboratory findings are also of interest:

October 20: Urine negative, specific gravity 1020.
November 27: Urine: albumin positive, blood positive with numerous red corpuscles, few leukocytes and epithelial cells.
November 30: Urine: albumin positive, with few granular casts, leukocytes and epithelial cells, but no red corpuscles.
December 7: Albumin negative, no red corpuscles.
December 13: Albumin positive, urobilin and urobilinogen positive.
December 14: White blood count 17,500
Polymorphonuclears 81
Monocytes 5
Lymphocytes 14

The autopsy on December 15, 1925, performed by Dr. Matras in the Pathological Institute of Professor Maresch, revealed the following:

There is a universal edema of the skin. The dura of the brain is tense, the meninges are somewhat thickened.

The fluid in the subarachnoid space is increased, with a slight edema of the brain. The ventricles are slightly enlarged and contain a clear fluid. The cord presents no macroscopic changes.

Hydrothorax and hydroperitoneum are present. Hemorrhagic infarcts are visible in both lungs, with a lobular pneumonia and fibrinous pleuritis in the lower lobes. The heart is enlarged, with eccentric hypertrophy of both ventricles, and dilated auricles. The valves are all normal. The myocardium is pale grayish red to yellow, and its consistency is reduced. Everywhere are small scars of whitish color in addition to the general fatty degeneration. The coronaries are straight and delicate, but along their course are numerous pinhead-sized whitish spots or nodules.

The aorta is smooth and appears entirely normal, as also its large branches. The thyroid appears normal. The liver is large, of normal consistency. On the surface are many irregularly outlined bluish red depressions 1 to 3 cm. in diameter and easily visible through the delicate capsule.

These areas are mostly in the left lobe and left half of the right lobe. On the cut surface they are dark red and depressed. The liver parenchyma is destroyed leaving behind a vascular network filled with blood. Here and there are thickened vessels with narrow or obliterated lumen.

The gall bladder appears normal. The spleen is enlarged. The kidneys are large, the capsule adherent and thickened. The surface is very irregular with numerous dark red retractions of various sizes. There are other small yellowish necrotic areas. On the cut surface the thickened arteries are visible, some appearing as round grayish areas without any lumen. The adrenal and pancreas appear normal.

On the arteries along the lesser curvature of the stomach, on the arteries of the small intestine in the immediate vicinity of the mesenteric attachment to the bowel, and on the vessels from the mesocolon to the large intestine are everywhere numerous nodules, often arranged in chains like a string of pearls (*perlschnurartig*). The nodules are mostly of the size of a hempseed. Some vessels present thickenings in the wall which hardly protrude beyond the surface. The mesenteric lymph nodes are somewhat enlarged. The prostate, testicles and epididymes appear normal. There are fresh thrombi in the veins of the prostatic plexus, in the posterior tibials and the muscle branches of the lower extremities.

In this case of acute periarteritis nodosa we find as the predominating symptom the polyneuritis, with paresthesias, muscle pains and weakness, anesthesia, loss of reflexes and wrist-drop. The

second most important symptom is the remittent temperature. Vomiting was the only gastric symptom. Then came signs of cardiac decompensation. The renal symptoms were the transient hematuria and albuminuria. Death resulted from cardiac failure with pneumonia and hemorrhagic infarction of the lungs.

The histological changes were typical of acute and subacute periarteritis nodosa of the heart, kidneys, arteries of the peripheral nerves, liver, and mesenteric branches.

CASE 3. *Clinical History:* Hugo H., 50 years old, entered the Wenckebach Clinic on August 19, 1925. The patient had measles, whooping cough, scarlet fever and diphtheria in childhood. At the age of 21 years he had a soft chancre. The patient denied the use of alcohol and was a moderate smoker.

The present illness began in June, about two months before his entrance into the hospital. He had an attack of angina with fever lasting ten days. He remained in bed three weeks and then returned to his work. After a four-day afebrile period he developed a second attack of fever lasting five days. Fourteen days later a third attack occurred. This began about the second of August and lasted until his admittance on August 19. His temperature during the attacks reached 39 to 39.5° C. During the third attack *pain and swelling of the legs appeared.* A week's rest in bed brought relief. *The pain was chiefly in the calves and in the peroneal muscles, making walking impossible.*

The examination on August 20, revealed the following:

A well developed man, with somewhat atrophic musculature. The temperature 37.7° C, respiration 20, pulse 90 and regular. The pupils were equal, reacted normally to light and accommodation. The tonsils were enlarged. The lungs presented nothing pathological, except for a dullness over the left apex. The heart was slightly enlarged to the left. The blood pressure was 150 systolic. A soft systolic murmur at the apex was heard on August 29, ten days after admission. The liver was slightly enlarged. The spleen was not palpable.

On both legs there were *areas of paresthesia involving chiefly the peroneal distribution.* In the center of these areas there was complete anesthesia. Here the pain was most intense a few weeks earlier.

Examination of the fundus oculi was negative. X-ray examination of the chest showed a darkening of the left apex, with calcified spots in the left hilum region. The heart showed an enlargement of the left ventricle. I found a hitherto undescribed anomaly of the aorta, a right-sided retro-esophageal aorta which was confirmed at autopsy. The blood and spinal fluid Wassermann were negative. Agglutination reactions for typhoid and paratyphoid were negative. The blood examination showed 12,000 leukocytes with 82 per cent polynuclear neutrophiles and 3 per cent eosinophiles. The red count was 3,600,000 and the hemoglobin 60 per cent, an index of 0.9. The spinal fluid was normal.

The examination of the patient failed to reveal the cause of his intermittent temperature which in the mornings reached as high as 39.3° C. The patient gradually developed an edema of both legs. The pulse was always rapid, averaging 110.

Several examinations of the urine revealed about 1/4 per 1000 albumin and numerous red blood cells and some leukocytes in the sediment. The residual nitrogen in the blood was normal.

Six days ante mortem the patient had a collapse, the pulse being 140, the temperature only 36.8° C. The next day râles were heard at the base of both lungs. Then dullness developed on both sides. The dyspnea became more severe and bronchial breathing was audible over the entire lung. The patient died on September 17, after a six-day fever-free period. The clinical diagnosis was sepsis of unknown cause with terminal afebrile pneumonia.

The postmortem examination performed by Dr. Feller in the Pathological Institute of Professor Maresch revealed the following:

A recent confluent lobular pneumonia of all the lobes, with a high grade pulmonary edema; eccentric hypertrophy of both ventricles, especially the left. The coronary arteries are normal except for slight atherosclerosis. There is a fatty degeneration of the myocardium. A right-sided aorta, which runs over the right bronchus and behind the esophagus, is found.

The liver displays on its surface numerous irregularly outlined dark red depressions which vary in size up to 2 cm. in diameter. Similar areas can be seen on the cut surface. The branches of the hepatic artery show marked periarteritis nodosa with occlusion of the small branches. The reddish depressed areas represent infarcts.

The kidneys are irregularly coarsely granular. The reddish gray depressions represent multiple healed infarcts. There are also many fresh anemic infarcts present. The branches of the renal artery show extensive changes with wall destruction, aneurysm formation, thickening of intima and thrombosis. Many are in the acute inflammatory stage. Those in the granulation tissue stage are often surrounded by a mantle of periarterial connective tissue. Some of the glomeruli present the typical picture of a glomerulonephritis. The pancreas and adrenal are macroscopically normal. The arteries of the arms and legs show miliary aneurysms on their smaller muscular branches. The arteries of the peripheral nerves show no typical changes macroscopically, but microscopic examination reveals marked changes.

The stomach and intestines appear normal. There are also no changes in the central nervous system.

When we briefly summarize this case we find: A man of 50 years suffered from an angina followed by three attacks of fever. The last attack was accompanied by edema of the legs with intense pain in the muscles, chiefly the peroneal group. He had a polymorphonuclear leukocytosis and intermittent temperature to 39.3° C, a nephritis with hematuria but no increased blood pressure. Death

was due to confluent lobular pneumonia and cardiac weakness. The postmortem examination revealed an acute and chronic periarteritis nodosa affecting chiefly the kidneys, liver, muscles of the extremities, and peripheral nerves. The presence of multiple infarcts in the kidneys in the absence of an endocarditis should always call to mind the possibility of periarteritis nodosa. We have diagnosed two cases postmortem by this finding, together with thickenings and nodule formations on the arteries.

CASE 4. *Clinical History:* Jacob S., aged 34 years had no children's diseases, and was never seriously ill until his present illness. The family history revealed nothing of importance. The patient's present illness began in June, 1924, five months before he came to the clinic. He had at the onset *generalized rheumatic symptoms* with temperature to 39° C. *There was no swelling of the joints*, but slight edema of the legs in the afternoon when the patient was up and about. He was under a physician's care and at home for seven weeks. He then went to a clinic where he remained four weeks on account of an acute nephritis and left feeling quite well. The rheumatic pains in the extremities disappeared. A few days after returning home the patient again developed the pains in the extremities with edema of the legs. Then severe headaches set in for about fourteen days. About two weeks after the onset of this attack an orchitis appeared. It lasted about four weeks. Since the development of the headaches the patient's vision has suffered. There were no mental disturbances. On account of the severe headaches the patient came to the clinic on November 17, 1924. The patient stated that he drank about one liter of wine daily, and smoked 30 to 40 cigarettes. He denied venereal infection.

The patient was a medium-sized well nourished man. He was somewhat stuporous. The skin was pale but not edematous. The pulse was 88, regular. The pupils were equal and reacted normally to light and accommodation. The thyroid was not enlarged, nor were any enlarged glands present in the neck region. The thorax was symmetrical, the lung borders normal. The heart dullness was slightly increased to the left. The aortic second sound was accentuated. The liver and spleen were not palpable. There was no ascites. The external genitals appeared normal.

Examination of the eyes on November 18, revealed the following: In the right eye there were no certain changes in the fundus. In the left there were variations in the caliber of the small arteries in the region of the papilla. There was a slight edema of the retina about the papilla. The residual nitrogen in the blood on November 20, was 34 mg. Examination of the urine showed albumin 2 per 1000 (Esbach) with erythrocytes, leukocytes and many granular casts in the sediment.

On November 27, the patient's headaches became more intense, with vomiting, sleeplessness and still greater reduction of vision. On December 3, the fundus showed: In the right eye the border of the papilla was indistinct, with marked edema of the retina, and small hemorrhages. No foci of retinitis were visible. There was an ablatio retinae in the nasal peripheral zone. The left eye showed more marked unsharpness of the papilla and edema around it. Many radiary hemorrhages were seen about the papilla. Ablatio retinae in the lower periphery. The residual nitrogen on December 5, was 50 mg.

Lumbar puncture yielded a pressure of 300 mm., the spinal fluid clear with 15 cells per cmm. The intense headache persisted. The patient had signs of cardiac failure with edema of the extremities and also râles at the base of both lungs. On December 10, the patient developed a deviation of both eyes to the left, unconsciousness and tracheal râles, and died.

The blood pressure of the patient varied between 170 and 190 systolic, and 110 and 120 diastolic. The concentrating power of the kidneys was reduced, the urine specific gravity never above 1017. The sediment contained red cells, leukocytes and casts, also renal epithelium. Once the stool gave a positive reaction for blood. The blood Wassermann test was negative. The temperature was subnormal mornings and reached 37.5° C afternoons. It was never above 37.5° C during the stay in the hospital.

The autopsy performed on December 10, revealed a chronic periarteritis nodosa affecting chiefly the kidneys, liver, heart, peripheral nerve vessels, and mesenteric arteries. There are numerous nodular thickening of the arteries. The most marked changes are seen in the coronaries, hepatic artery branches, renal arteries and mesenteric arteries.

The myocardium is macroscopically unchanged. There is a marked hypertrophy of the heart, especially the left ventricle. The liver presents numerous smaller and larger depressed and irregularly outlined gray-red areas, which consist almost entirely of blood capillaries and in which the liver parenchyma is destroyed.

The kidneys show the most marked changes. The surface presents relatively small light gray and grayish yellow, flat prominent smooth areas with irregular outline. These represent the rests of the cortex. Between these areas are numerous dark red depressions representing the healed infarcts due to arterial occlusion. The larger branches of the renal artery in the hilum region are greatly thickened. The testicles contain a number of smaller and larger fibrous scars. The vessels of the peripheral nerves *show no macroscopic changes.* The cerebral vessels appear macroscopically unchanged, yet in the region of the left caudate nucleus there are several grayish red unsharply outlined and slightly depressed areas. Also in the cerebellar cortex there are multiple foci of hemorrhage of various size up to 2 cm. Bilateral pleural effusion and uremic pericarditis are also present.

Histologically we find the vascular thickenings to be due to a thickening of all the layers of the wall, especially the intima and adventitia with rich perivascular connective tissue formation. In some places acute inflammatory changes are still present, with

leukocytes and a few eosinophiles. Many arteries are occluded by thrombi undergoing organization. Most of the nodules are in the granulation tissue stage.

The pathological diagnosis is: Periarteritis chronica nodosa; atrophia renis ex periarteriitide. Uremia.

In this case also the pains in the extremities, without any joint involvement dominated the clinical picture. An attack diagnosed acute nephritis followed two months after the onset of the disease. Then came an attack of orchitis, severe headaches and marked visual disturbance. The changes in the fundus are of great importance because their occurrence enables the ophthalmologist acquainted with periarteritis nodosa to make the diagnosis.

The hematuria and terminal uremia are quite characteristic of this disease. The hemorrhages in the central nervous system are unusual and have been seen in only nine cases to date. As in this case the diagnosis of acute nephritis is often made in the early stage because of hematuria.

CASE 5. *Clinical History:* The patient, Joseph S., entered the hospital on August 11, 1922. There was nothing of importance in the family history. The patient was never sick until 1918, when he became suddenly ill with *a high fever, severe icterus and "acute nephritis."* He was then in a hospital for six weeks and gradually recovered and returned to his work.

The present illness began in May, 1922 (four years later) with cramp-like pains in the epigastrium. These pains were so intense that the patient was forced to bed. He had a feeling of pressure in the stomach region, which was intensified by the taking of food. It was relieved by hot applications. There was no vomiting. Appetite was poor. The patient was constipated. Several weeks' treatment by a family physician was without effect and the patient entered the clinic on August 11, 1922.

The patient was much emaciated, the musculature atrophic. The skin was brown and pigmented. The mouth mucosa was not pigmented. The pupils reacted normally to light and accommodation. The veins of the neck were somewhat dilated.

A slight asymmetry of the thorax was present. The lungs were normal. The heart was slightly enlarged to the right; the heart sounds were normal. The pulse was regular, 90 to 100 per minute. The blood pressure was 150.

The abdomen was retracted. The spleen was not palpable. The liver could be palpated below the costal margin and seemed to have an irregular border. The patient complained of intense pain in the epigastrium.

On August 15, the patient revealed a dullness at the base of both lungs with signs of bronchitis. The apex beat was two finger-breadths outside the midclavicular line. On August 24, a marked edema of the legs had developed, as well as a generalized anasarca. There was dyspnea, the pulse was 120. The bilateral hydrothorax was increased. There was a systolic murmur at the apex of the heart. *In spite of digitalis therapy* the patient's edema increased and the quan-

tity of urine decreased. The edema of the legs was relieved by puncture but soon recurred. On October 5, the patient became very dyspneic with marked congestion of the veins of the neck. The dullness over both lungs reached to the fourth rib. The patient died on October 11, 1922.

At no time during the patient's stay in the hospital was there any fever, the highest temperature being 36.8° C. At the same time the pulse was always 100 or more.

The following laboratory findings are of interest:

The stomach contents was anacid. The stool was positive for blood. The urine contained 1/4 per 1000 albumin, but no erythrocytes or leukocytes. The specific gravity was low, the chlorides reduced. The Wassermann was negative. The fundus oculi was examined October 7, and reported normal.

The autopsy was performed by Dr. Feller on October 11, 1922. The lungs are free from tuberculosis. There is a marked compression atelectasis of both lower lobes, with slight emphysema of the upper lobes. The lungs are congested. The heart is hypertrophic, especially the left ventricle. The subepicardial fat is absent. The coronary arteries are thickened. Numerous nodular thickenings are seen in the wall of both coronary arteries. On cross-section the wall appears greatly thickened, the lumen reduced to a narrow slit in places.

The aorta is practically free from atherosclerosis. A thrombus is present in the left auricle.

The liver is relatively small, and resembles a hepar lobatum syphiliticum. Its surface is coarsely granular, and in a number of places there are deeply penetrating depressed scars. The capsule of the liver is wrinkled in the sunken areas. The left lobe is small and more markedly affected than the right. On the cut surface are found septa of connective tissue containing obliterated thickened branches of the hepatic artery. Nodular thickenings occur on some of the branches. There are also areas of normal liver tissue with normal acinous structure; and immediately adjacent are areas of marked congestion in which the parenchyma has disappeared. The spleen is slightly enlarged. The pancreas is atrophic, but its lobular structure is well preserved.

The kidneys are about normal in size. The arteries are thick-walled and gaping. The surface is very irregular with numerous depressions of various size. These are reddish in color. On the cut surface are partly wedge-shaped and partly more rounded elevations which correspond to the nodules of parenchyma seen on the surface. In the retracted areas the cortex is absent. Everywhere

the branches of the renal artery are thickened, some have nodules. Many are totally obliterated. Some of the nodules are spindle-shaped and surround the entire circumference, others are smaller and involve only part of the circumference. In places linear thickenings appear in the wall.

The mesenteric arteries are rigid and gaping with scattered small nodules in the wall. In the testis are several large infarcts. The gastric arteries are thickened and rigid, some show no visible lumen.

The histological examination of over fifty blocks of tissue from various organs revealed the fact that we are dealing here with the *histologically healed end-stage* or *scar tissue stage* of a *generalized periarteritis nodosa*, a *periarteritis obsoleta nodosa*. Nowhere is there evidence of acute inflammation. In practically all the cases hitherto described various stages of the inflammatory disease have been found, due to the acute exacerbations so common in this disease.

The characteristic changes found in this case are:

(1) Intima proliferation with new formation of elastic fibrils, leading to stenosis or even complete occlusion.

(2) Extensive destruction of the media including the elastica interna, or of the entire vessel wall, with aneurysm formation and thrombosis. The thrombosis is followed by complete organization, with here and there deposition of hemosiderin.

(3) A periarterial healed granulation tissue mantle consisting of dense fibrous connective tissue containing capillaries and hemosiderin deposits.

(4) Extensive destruction with even aneurysm formation in arteries with high grade intima proliferation.

(5) Healed infarct scars in most organs.

(6) High grade stenosis of both coronary arteries.

The consequence of the periarteritis obsoleta nodosa in our case was the development of: contracted kidneys, hepar lobatum, high grade coronary stenosis, infarction of the testicle, myomalacia scars, etc.

Death resulted from myocardial and renal insufficiency. Of great interest is the fact that the patient had no symptoms of angina pectoris, although both coronary arteries were reduced to one-fourth or less of their normal caliber. This finding shows us that a reduction of the blood supply to the myocardium need not cause angina pectoris. Perhaps the rigidity of the arteries due to their

great thickness, which made spasm of the vessels impossible, explains the absence of angina pectoris in this case.

We have here a new clinical syndrome due to histologically healed periarteritis nodosa: renal insufficiency, cardiac insufficiency and hepar lobatum. The finding of hepar lobatum in this disease makes it necessary to examine the vascular changes in such livers more carefully, instead of assuming the syphilitic nature of this condition. Also extensive fibrosis or atrophy of other organs may be due to periarteritis nodosa.

The Important Symptoms Observed in the Five Cases Herein Reported

Accelerated regular pulse	5
Edema of the legs	5
Septic type of temperature	4
Pain in the extremities, polyneuritis (wrist-drop in two cases)	4
Hematuria	4
Cardiac insufficiency	3
Melena	3
Cerebral symptoms	2
Onset with acute angina	2
Abdominal pain	2
Changes in the fundus oculi	1

Summary

1. Periarteritis nodosa is a specific infectious disease probably caused by a filterable virus, with an elective affinity for the arteries of the body. The organs most commonly involved are the kidneys, heart, liver, muscles, peripheral nerves and gastro-intestinal tract. Any organ, or all may be affected.

2. The chief symptoms are a septic temperature, polyneuritis and polymyositis, hematuria or nephritis, abdominal cramp-like pains, progressive emaciation. The great variability of the symptoms, pointing to involvement of various organs, and the tendency toward acute exacerbations are suggestive of periarteritis nodosa.

3. The pathological changes in the arteries may be divided into four stages: (1) alterative-degenerative, (2) acute inflammatory, (3) granulation tissue, (4) histologically healed end-stage or scar tissue stage.

4. We have described the histologically healed end-stage of periarteritis nodosa. A patient with a single severe illness consisting of

icterus, high fever and acute nephritis died four years later of renal and cardiac insufficiency. The postmortem findings revealed a histologically healed end-stage of periarteritis nodosa affecting all the organs of the body except the central nervous system. The contracted kidneys, hepar lobatum, myomalacia scars, pancreatic and adrenal atrophy, and coronary stenosis were all due to this disease.

5. A new clinical syndrome characterized by cardiac insufficiency which failed to react to digitalis, renal insufficiency with low specific gravity of the urine and reduced chlorides, progressive emaciation, abdominal pain and hepar lobatum is described. Especially important is the fact that the patient lived four years after his single acute attack, and that the patient was entirely free from temperature during his fatal illness. The absence of temperature indicates histological healing of the disease.

6. Periarteritis nodosa is of interest to the surgeon because it can produce the symptoms of an acute cholecystitis with severe changes in the gall bladder, internal hemorrhage due to rupture of an aneurysm (kidney, liver, pancreas, brain, gastro-intestinal tract, lungs), or gangrene of the intestine with peritonitis. The great frequency of polyneuritis as the first and predominating symptom should interest the neurologist. In four of our five cases this was a prominent symptom. And the ophthalmologist who becomes acquainted with the disease may enable us to make the correct diagnosis *in vivo* by finding nodules or localized thickenings on the retinal arteries.

7. The diagnosis of periarteritis nodosa is very difficult, and it is only by keeping in mind the cardinal symptoms which we have described that the internist will be able to recognize the disease, after having ruled out other possibilities. In a few cases the diagnosis has been made by finding nodules in the skin with the characteristic histological changes in the blood vessels.

8. I wish to call attention to the fact that there is a microscopic form of periarteritis nodosa which can be recognized only by a careful study of tissues, especially with elastica stains of the blood vessels. Pathologists should examine carefully the arteries in atrophic organs or those with extensive fibrosis not to overlook changes such as we have described for the end-stage of periarteritis nodosa. It is possible that some cases of insufficiency of one or more glands of internal secretion may be due to atrophy caused by this disease. We

have seen changes in the thyroid, adrenal, pancreas, ovary, and testis due to periarteritis nodosa.

9. How often periarteritis nodosa of a single organ or of numerous organs, comes to a complete standstill is difficult to say. We are inclined to consider complete histological healing, as in one of our cases, a rare occurrence. For in practically all the cases till now described acute as well as chronic changes have been present.

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DESCRIPTION OF PLATES

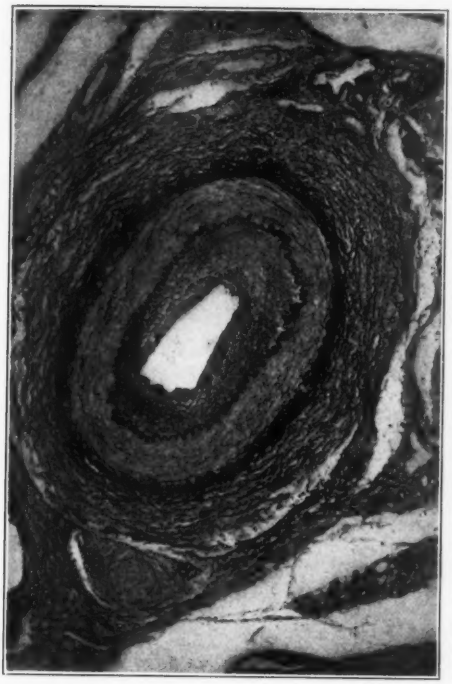
PLATE 89

FIG. 1. (Case 5.) Healed periarteritis nodosa. A branch of the mesenteric artery with marked intimal proliferation with new formation of elastic fibrils. At this level the internal elastic layer is intact. Serial sections showed areas of wall destruction from which the intimal proliferation progressed. × 150.

FIG. 2. (Case 5.) Left coronary artery. This vessel presents an extensive destruction of about two-thirds of the circumference of the artery. The lumen has been greatly reduced in size by organized thrombi and intimal proliferation. There is also the characteristic periarterial healed granulation tissue mantle. Elastic tissue stain. × 100.

FIG. 3. (Case 5.) Pancreatic artery. Here the high-grade intimal proliferation predominates. At the other levels characteristic wall changes were found. Other arteries with complete obstruction by organized thrombi or intimal proliferation were found. × 150.

FIG. 4. (Case 5.) A bronchial artery at the lung hilum. This vessel presents remarkable changes due to wall destruction with aneurysm formations, high-grade intimal proliferation and final central thrombosis with organization and recanalization. Elastic tissue stain. × 200.



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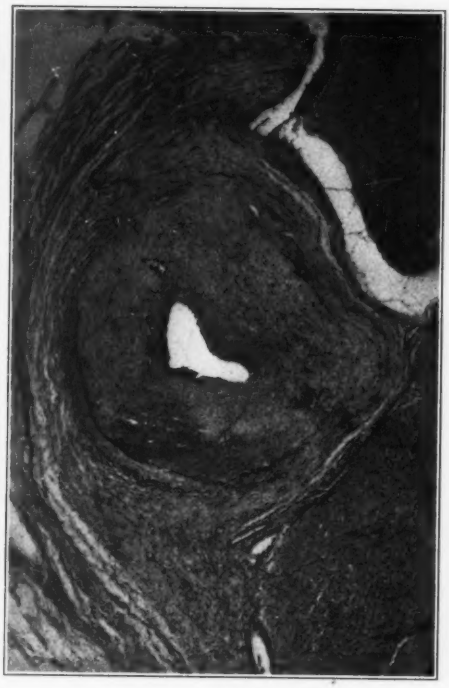

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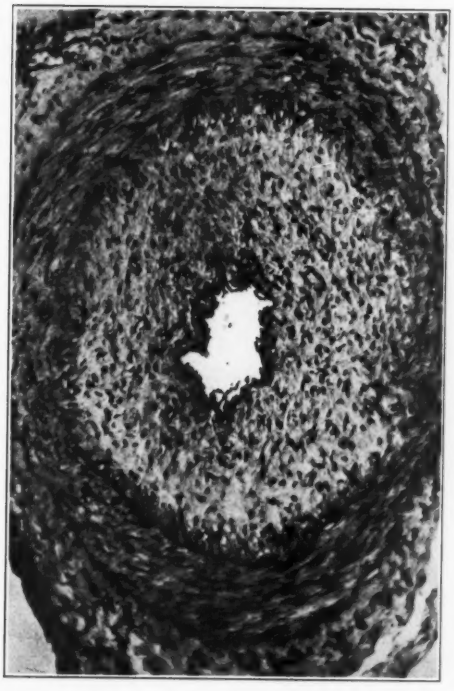

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4

Arkin

Periarteritis Nodosa

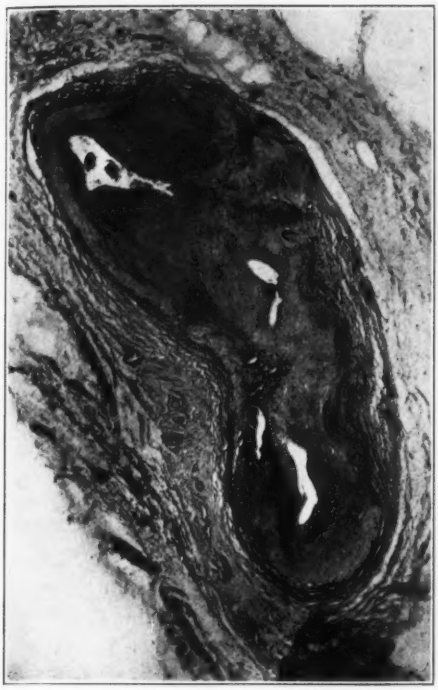
PLATE 90

FIG. 5. (Case 5.) Para-esophageal artery, showing extensive destruction of the wall with aneurysm formation and complete organization. There is also stenosis of the lumen due to thrombosis with organization, and intimal proliferation. Elastic tissue stain. × 150.

FIG. 6. (Case 5.) Hepatic artery in healed periarteritis nodosa. Note the almost complete destruction of the artery, with organized thrombosis of the lumen, and a very thick periarterial vascularized healed granulation tissue. The acute changes must have been very extensive. × 100.

FIG. 7. (Case 5.) Liver in healed periarteritis nodosa. Note the large area of liver cell destruction, with beginning regeneration from some of the bile ducts. The branches of the hepatic artery show very marked changes, with total occlusion of many. The resulting infarction with organization produced deep scars with wrinkling of the liver capsule. The gross appearance resembled that seen in hepar lobatum syphiliticum. Hematoxylin and eosin. × 75.

FIG. 8. (Case 5.) Older infarcts of adrenal due to periarteritis nodosa. Only a small zone of cells at the periphery appears normal. × 150.



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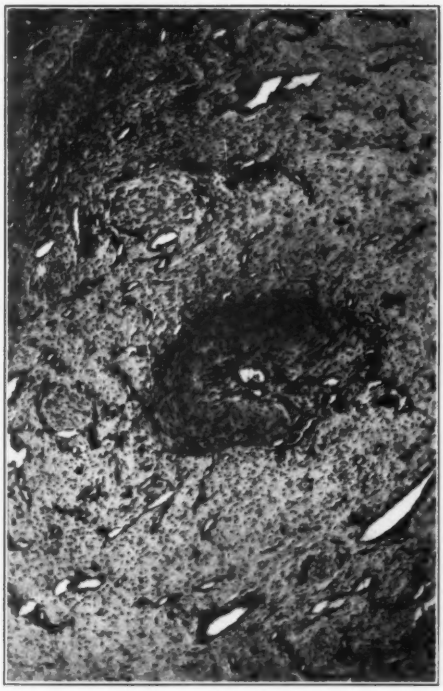

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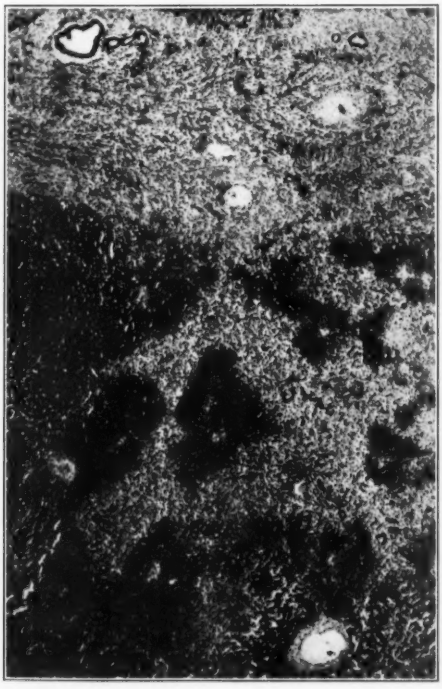

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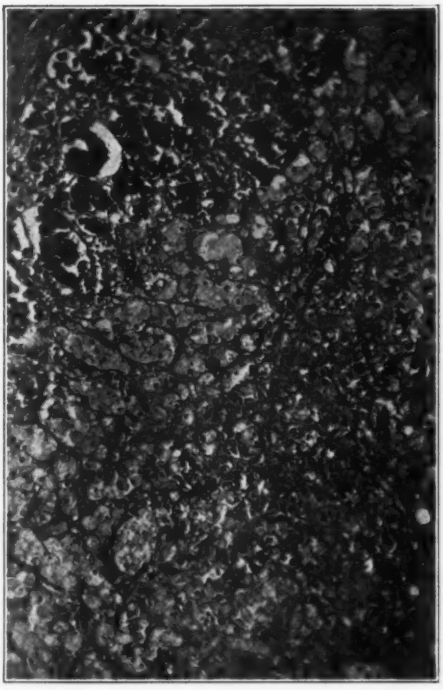


8

Arkin

Periarteritis Nodosa

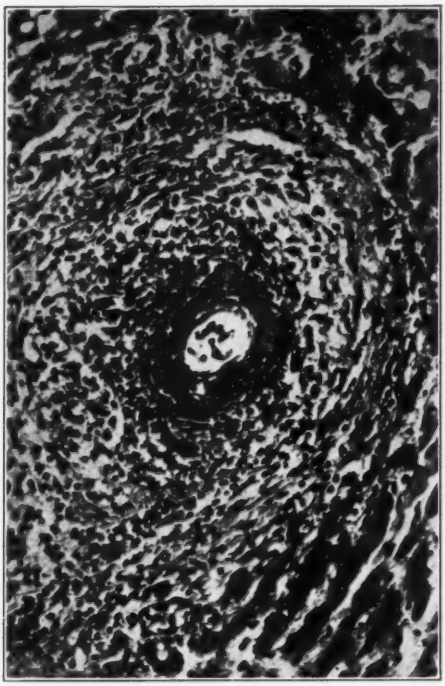
PLATE 91

FIG. 9. (Case 1.) Acute periarteritis nodosa of the liver. This is the very early acute stage with hyaline necrosis and fibrinous exudation. There is some edema and cellular infiltration. × 200.

FIG. 10. (Case 1.) Acute periarteritis nodosa of the brain. This small arteriole reveals only a periarteriolar hemorrhage without any cellular infiltration. In other sections similar hemorrhages with wall changes were found. × 300.

FIG. 11. (Case 1.) Acute periarteritis nodosa of the sciatic nerve. This small artery reveals a very early acute stage, with fibrinous exudation and slight cellular infiltration. The process is in the media and subintimal connective tissue. The lumen has become V-shaped as a result of the exudation with elevation of the endothelium. This finding explains the eccentric lumen so often found in the arteries of the healed case. × 200.

FIG. 12. (Case 1.) Acute periarteritis nodosa of the kidney. This section shows an aneurysm formation, with thrombosis and early organization. The acute inflammation has subsided. Hematoxylin and eosin. × 50.



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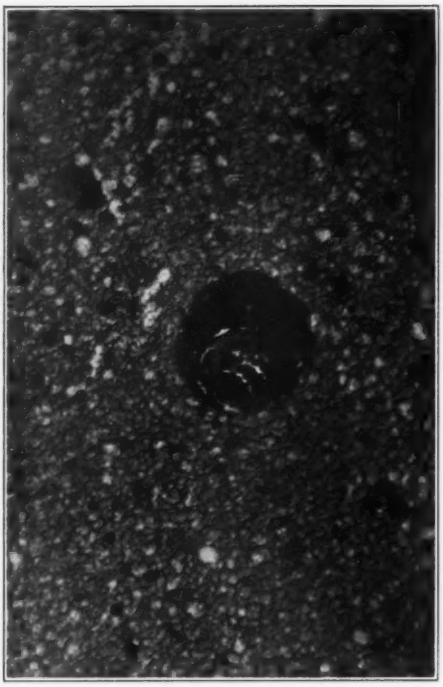

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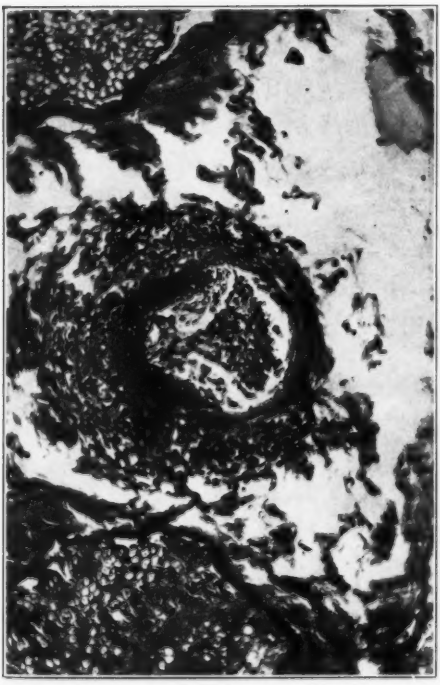

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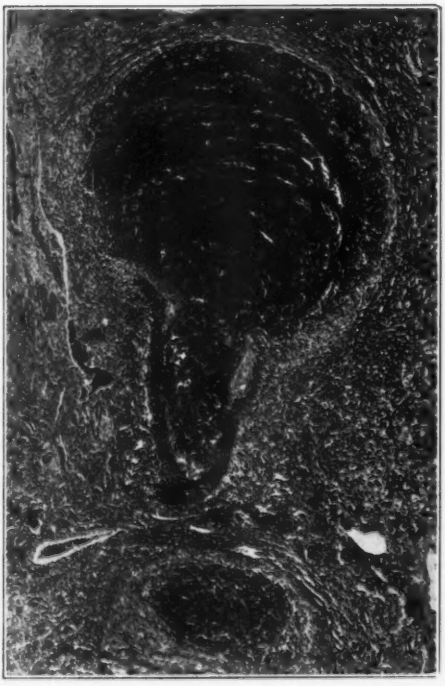
Arkin



12

Periarteritis Nodosa

PRIMARY SYMPATHICOBLASTOMA OF THE SKIN OF THE THIGH *

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INTRODUCTION

Since Wright [1] in 1910 first drew the attention of the medical world to the sympathetic neuroblastomas and reclassified the so-called round-celled sarcomas of the suprarenal medulla reported previously, many more tumors of this type have been recorded. At that time he stated that these growths could not be very rare as he himself observed five cases within one year. Boyd [2] encountered his three cases within a few months of one another. The three cases of Kwartin and Twiss [3] occurred within eighteen months. Yet in 1915, in an analysis of 2000 cases of malignant neoplasms in the young, Warthin [4] made no mention of the neuroblastoma. Saphir's case [5] was the only one among 3950 autopsies. The records of the pathological laboratory of the Albany Hospital for the last thirty years do not reveal a case of sympathicoblastoma. So far as we have been able to determine, a sympathicoblastoma of the skin of the thigh, primary or metastatic, has not yet been reported. For this reason the following case is of especial interest. We are indebted to Dr. C. F. Kivlin of Troy, New York, and Dr. A. E. Houle of Cohoes, New York, for the use of the clinical records of the patient.

CASE REPORT

Clinical History: B. H., white, male, 9 months old. A paternal aunt died of carcinoma of the rectum, a maternal grandmother of cancer, the organ affected not being known.

His past history was quite uneventful. He was breast-fed. He slept, nursed, and digested his food well. The bowels were regular. He was normally developed for his age and well nourished, but the skin and mucous membranes were somewhat pale. Examination of the chest and abdomen revealed nothing abnormal.

On November 8, 1927, a small extravasation of blood appeared spontaneously on the anterior aspect of the left thigh. This area was about the size of a cherry, freely movable, painless to the touch, and evidently caused no pain, as the infant seldom, if ever cried.

*Received for publication February 21, 1930.

By February 14, 1928, the small bloody tumor had assumed larger proportions. It was still freely movable, sharply outlined, and hard to the touch. There was no inguinal lymphadenopathy. A clinical diagnosis of hemangioma was made and the mass removed by Dr. C. F. Kivlin at the Troy Hospital. It was about 4 cm. in diameter, encapsulated, and contained many hemorrhagic areas as well as foci of pale, cellular appearing tissue. The pathological report was sympathicoblastoma, made by one of us (V. C. J.).

In August 22, 1928, the child was again operated on by Dr. Kivlin, for a recurrence of the tumor at the same site in the thigh beneath the scar. Physical examination of the rest of the body was negative.

Gross Description of the Recurrent Tumor: The specimen consisted of an ovoid mass, 8 by 3 by 3 cm., and a smaller one of about the size of a cherry. The larger growth lay subcutaneously but was visible externally as a red prominence. On cross-section, they were all hemorrhagic and spongy with very cellular, granular areas, grayish to opaque yellow in color (Fig. 1). They appeared entirely encapsulated and showed no demonstrable relationship to any vessel or nerve.

Microscopic Findings: One-half of the gross specimen was fixed in Zenker's solution and the other in 10 per cent neutral formalin. Paraffin sections were stained with hematoxylin and eosin, Van Gieson's picric-acid fuchsin, Mallory's phosphotungstic acid hematoxylin, Foot and Mènard's silver stain and Laidlaw's silver stain. Also, blocks were treated by Levaditi's method for myelinated nerve fibers.

All the sections are very cellular, with large and small hemorrhages scattered throughout. Thin strands of connective tissue group the tumor cells into variously sized alveoli. The tumor cells are round and about the size of small lymphocytes. They consist almost entirely of a hyperchromatic nucleus with a very narrow rim of faintly staining cytoplasm, scarcely discernible in many of the cells. Intimately connected with these tumor cells are numerous fine fibrillae, which stain faintly blue with hematoxylin and eosin, yellowish brown with the Van Gieson stain, dull blue with Mallory's phosphotungstic acid hematoxylin stain, and colorless with the different silver stains (Fig. 2). In other words, these fibrillae do not give the characteristic tinctorial reactions for connective tissue or neuroglia. Intermixed with these smaller tumor cells with dense hyperchromatic nuclei, are numerous slightly larger cells with a larger and less dense nucleus, surrounded by a larger amount of still faintly staining cytoplasm. These resemble the more differentiated

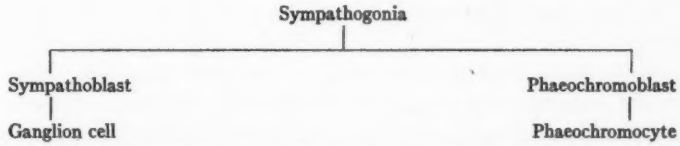
sympathoblast in contrast to the previously described sympathogonia or *bildungszellen*. As stained by Laidlaw's method, the nuclei of these tumor cells are all silver-positive, which is in agreement with Laidlaw [6] who stated that ectodermic cells retain the silver stain. There is no rosette formation but in places a tendency to sheaf-like arrangement. No ganglion cells are present anywhere. Collagen and reticulum are found only in the connective tissue septa of the tumor and in the walls of blood vessels.

The tumor cells have invaded the connective tissue capsule and infiltrated the surrounding fatty tissue. Even the dense fibrous layer just beneath the skin has become sparsely dotted with tumor cells. However, the various sections show that the neoplasm had been removed with a wide margin.

Sections were submitted to Dr. S. B. Wolbach of the Harvard Medical School, who concurred in the diagnosis of sympathicoblastoma.

Discussion

When it is considered that the primitive nerve cells migrate from the neural crest to different parts of the developing embryo to form the future sympathetic system, any dysontogenetic factor may cause these primitive cells to become arrested anywhere in the body, later to develop into the various tumors derivative of the sympathogonia. The embryological development of the sympathogonia may be represented in the following schema (after Poll):



As regards the right branch of the schema, Rabin [7] has very recently made an excellent review of the benign phaeochromocytomas of the suprarenal medulla.

Sympathetic neuroblastomas have been observed elsewhere in the body than in the suprarenal glands. Landau,[8] Anitschkow,[9] Boyd,[2] and Wollstein [10] reported these growths as originating in the retroperitoneal sympathetics. Pick [11] and Lemeland and Durante [12] observed a "sympathoma embryonale" of the uterus.

Two of Harbitz's cases [13] were in the sacral region. Alezais and Imbert [14] found their tumor connected with the glandula coccygea. In the cases of Wright,[1] Anderson and Shennan,[15] and Nieden,[16] the growths were situated in the thoracic cavity. In Martius' case,[17] the tumor originated in the cervical sympathetic and extended down into the thoracic cavity, completely surrounding the superior vena cava and the vena azygos. Capaldi's second case [18] is very similar in that the sympathicoblastoma of the cervical sympathetic extended down into the thoracic cavity and also invaded the spinal canal from the medulla oblongata to the eighth dorsal vertebra. His first case is very unique in that there were three primary tumors — one in the left inferior cervical sympathetic ganglion, another in the right inferior cervical ganglion, and the third in the retro-adrenal sympathetics. All three of these tumors invaded the spinal canal by direct extension. In Dunn's case,[19] the tumor was located over the right temple. Ritter [20] reported two case of neuroblastoma of the jejunum. MacNaughton-Jones and Turnbull [21] described a large ganglioneuroma (partly neuroblastomatous) of the mesentery. In Cushing and Wolbach's case,[22] the tumor was situated in the mid-scapular, right paravertebral region. The cases of Anitschkow, Capaldi, and Cushing and Wolbach are interesting in that the growths penetrated into the extradural spaces of the spinal canal through the intervertebral foramina, producing hour-glass tumors. Symmers [23] described a recurrent neuroblastoma of the scapular region although he mentions that, since the patient was lost sight of, its derivation from the adrenal capsule cannot be disclaimed. In the case here reported, the tumor was located in the skin of the thigh.

In addition to its origin from the sympathogonia, a neuroblastoma can theoretically arise as part of a teratomatous or teratoid process and thus be located anywhere along or near the midline where toti- or multipotential cells are liable to become misplaced. In one of Harbitz' cases, the finding of embryonic cartilage among the tumor cells of a neuroblastoma in the sacral region indicates that such an occurrence may have taken place there. In Suzuki's case [24] of a sympathetic adrenal tumor, fat cells of the signet ring type were also present. An overgrowth of the neuroblastoma cells or of any neoplastic tissue can efface most or all of the other identifying structures of the teratoma.

That neuroblastomas of the sympathetic may sometimes occur in the adult is shown by the recent papers of Meltzer [25] and of Blumensaat,[26] who collected four cases in addition to one of his own. The above-mentioned cases of Symmers, Ritter, and Lemeland and Durante were in adults. However, in general, the malignancy varies inversely with the age of the patient; that is, sympathicoblastomas are more common in the young and ganglioneuromas more common in adults. According to Pick and Bielschowsky,[27] we have the immature neuroma (neuroblastoma) and the mature neuroma (ganglioneuroma) depending on the varying degree of differentiation of the embryonal neurocytes.

Thus, microscopic findings have shown that all gradations and admixtures exist from the highly malignant tumor consisting mostly of the sympathetic *bildungszellen* type to the very bengin tumor consisting wholly of ganglion cells and nerve fibers with or without the sheath of Schwann cells. Wright,[1] Harbitz,[13] Dunn,[28] Lehman,[29] Wolbach and Morse,[30] Saphir,[5] Matzdorff,[31] and others have described cases where the tumor cells were of the sympathoblast type, intermingled with the characteristically staining fibrillae with or without Kuester's rosettes. In the cases of Anitschkow,[9] Monro and Dunn,[32] Dunn,[19] Bülbring,[33] Wollstein,[10] and others, neuroblastomatous and ganglioneuromatous areas were intermingled; or the two parts may be distinct but connected together as in the case of Martius.[17] In the so-called malignant ganglioneuromas, it is the neuroblastomatous portions that gives rise to metastases. All three elements — neuroblastomatous, ganglioneuromatous and paraganglioneuromatous — were represented in the tumors reported by Hedinger,[34] Suzuki,[24] Wahl,[35] and Glomset.[36] There are already on record numerous examples of ganglioneuroma distributed throughout the body but very rarely intracerebrally. Some of the unusual cases are those of Knauss [37] and of Kredel and Beneke,[38] where the subcutaneous nodules of ganglioneuroma totalled over 60 and 160 respectively. Hagenbach's paper [39] is of special interest in that the ganglioneuroma in his case occurred in the region of the knee joint. It is to be noted here that the sympathicoblastoma in our case was located under the skin of the anterior aspect of the thigh. Although a malignant sympathetic tumor has not yet been observed in this location, the more highly differentiated tumor nodules observed subcutaneously in the cases of Knauss, Kredel and Beneke, and

Hagenbach lend support to our belief that a primary origin of the sympathicoblastoma in the skin of the thigh is not improbable. Our patient at the present writing is a vigorous child, almost two years after the removal of the recurrent growth, apparently normal in every way.

Summary

A case of sympathicoblastoma, primary in the skin or subcutaneum of the thigh of a nine-months-old infant, is here reported. There was a recurrence in the same location within six months. So far as we have been able to determine, this is the only instance of a sympathicoblastoma, occurring in such an unusual location. At the present writing, almost two years after the removal of the recurrent tumor, the child is vigorous, robust, and apparently normal in every respect.

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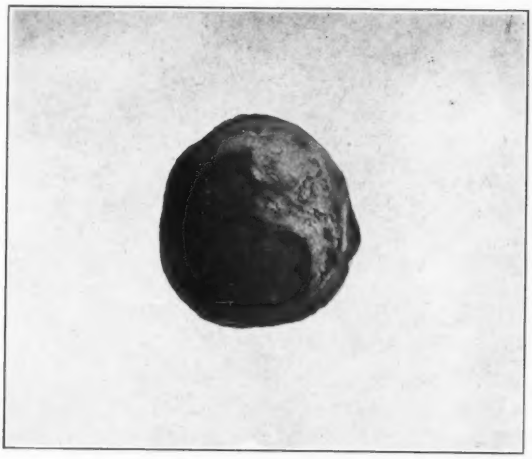
DESCRIPTION OF PLATE

PLATE 92

FIG. 1. Photograph of a cross-section of the recurrent tumor of the skin of the thigh (after formalin fixation). The large dark area is due to marked congestion and hemorrhage. Necrotic foci in the lighter right quadrant. × 1.4.

FIG. 2. Photomicrograph showing the admixture of tumor cells of the sympathogonia and sympathoblast types and the fine fibrillary intercellular substance. Phosphotungstic acid hematoxylin. × 778.



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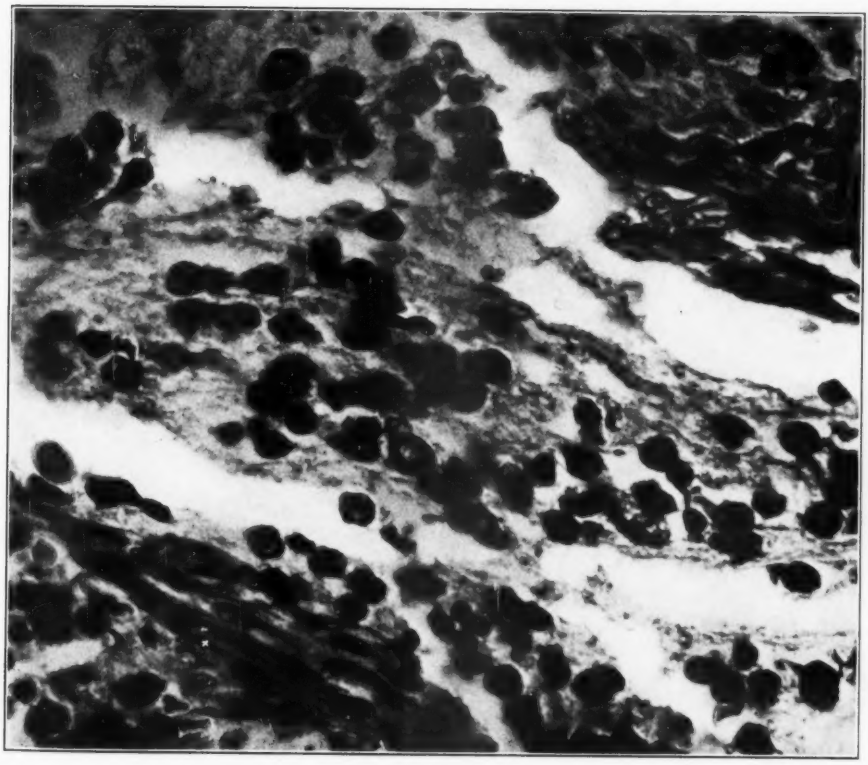

2

Jacobsen and Hosoi

Primary Sympathicoblastoma of Skin of Thigh

SILVER STAINING OF THE ENDONEURIAL FIBERS OF THE CEREBROSPINAL NERVES *

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The point of departure of any study of the connective tissue sheaths of the peripheral nerve must still be the work of Ranvier [1,2,3] and of Key and Retzius.[4,5] Review of recent literature shows scarcely a line added to the descriptions given by these masters fifty years ago. The endoneurium is described by the one as strands of fibrous connective tissue, by the other as a sheath, lying in immediate contact with the individual nerve fiber. Obviously, the endoneurium consists of cells as well as fibers: we shall consider the fibers only.

The fibers of the endoneurium are revealed best by the silver methods that have been devised for the study of collagen; in the writer's opinion, the best of them is his modification [6,7] of Hortega's technique described in a former number of the Journal. We shall proceed to apply this method to the endoneurial fibers of a cerebrospinal nerve.

THE DISTAL NERVE

As shown in Fig. 1, the endoneurial fibers of a distal nerve are arranged in two distinct patterns. There are longitudinal collagen fibers, and there is a delicate web around each nerve fiber.

The longitudinal fibers have been described by many authors. They constitute the *Fibrillenscheide* of Key and Retzius (1873,[4] page 354; 1876,[5] page 101), and the intrafascicular connective tissue of Ranvier (1875,[1] page 764; 1889,[2] page 585). Running between and over the nerve fibers, these longitudinal collagen fibers form a coarse network of meshes elongated in the direction of the nerve (Nageotte[8,9]).

The longitudinal fibers may be demonstrated fairly well by any good collagen stain but, like all fibers of the connective tissue group, they are brought out more effectively by silver. Stained with silver, they were described and illustrated by Ramón y Cajal (1909,[10] page 264; 1913,[11] page 76; 1928,[12] page 63).

* Received for publication April 7, 1930.

The web sheathing the individual nerve fiber is revealed only by silver and only by silver used in a particular way. It has been seen by few. Studnička,[13] Snessarew,[14] and Ranke [15] note in passing that their respective silver techniques reveal "a network in the Schwann sheath and in the capsules of the ganglion cells." However, Plenk,[16] (1927, page 380), of the Histological Institute of Vienna, was really the first to describe and illustrate this delicate network which dips in at Ranvier's nodes and forms a closely fitting sheath around each nerve fiber. In the summer of 1928, Herr Plenk had the kindness to show me his preparations and to look at mine. I believe that it is fair to say that we agreed that the technique described in this paper gives the clearer view of the web. Plenk's work is a mine of information about argyrophil webs in all parts of the body and there is a full bibliography, from which the references in this paragraph were taken.

Here and there, the longitudinal fibers give off branches to the web and, at points where the nerve fibers have been torn apart, delicate filaments may be seen to pass from one web to another; these observations were made first by Nageotte.[9] While we have dealt with them separately for the purpose of description, longitudinal fibers and web undoubtedly form a whole and are to be regarded as the ultimate distribution of fibrous connective tissue around the individual nerve fiber.

We have found the same construction of the endoneurium in man, and in all of the laboratory animals examined, cat, dog, rabbit, rat, monkey and guinea pig.

The Dorsal Root Ganglion

From the fibrous capsule of the ganglion, septa of fibrous connective tissue extend inward, forming sheaths around each nerve fiber and around each ganglion cell. All this is demonstrable easily by the usual collagen stains; but, at this point, silver takes up the tale and reveals around each ganglion cell a closely plaited web of argyrophil fibers, as shown in Fig. 2. Here and there on the web are coarse, parallel fibers, representing the longitudinal fibers of the endoneurium.

In these argyrophil webs, there seems to be only one orifice, the point of exit of the axis cylinder. Fortunate sections show the argyrophil fibers woven neatly and smoothly around this orifice and

at this point the argyrophil web of the ganglion cell is continuous with the argyrophil web of the nerve fiber.

Over the ganglion cell, the web is stronger and bolder than on the distal nerve. We shall see it become still denser and more intricate as we follow the root upward to and into the cord.

Glomeruli

Immediately on leaving the ganglion cell, the axis cylinder describes a curious convolution known as the glomerulus. The argyrophil web clings closely to the nerve fiber and accompanies it in all of its twistings and turnings. In the glomerulus, heavy argyrophil fibers run around and around the nerve fiber, forming a tubular sheath which gives an impression of resistance and rigidity. The arrangement suggests the spiral wire reinforcement around a rubber garden hose. Counterstaining with azo carmin shows the winding axis cylinder inside of the argyrophil tube.

The Spinal Nerve Roots

Fig. 3 shows a sensory root entering the cord. In the roots, both the longitudinal fibers and the web are heavier and more prominent than in the distal nerve. Here the plexus arrangement of the endoneurium is particularly evident, heavy argyrophil fibers crossing the nerve bundle in all directions. As in the distal nerve, at points where the nerve fibers have been torn apart, delicate web filaments are seen to pass from one nerve fiber to another, (Nageotte[9]).

The Pia

Silver brings out in striking contrast the two layers of the medullary pia described by Key and Retzius [5] (1875, page 143). As the root joins the cord, it is seen clearly that endoneurium and perineurium are merely peripheral extensions of these two layers of the pia mater. The outer layer of the pia consists of concentric laminae of dense collagen; it continues out over the root as the laminated perineurium.

The inner layer, the intima piae of Key and Retzius, is a loosely woven network of collagen and reticulum fibers that splits into two layers to surround the roots. The outer layer of this intima piae extends out over the root just beneath the perineurium. In fact, it may

be said that the peripheral nerve never succeeds wholly in getting outside of the pia mater. In cross-sections of such trunks as the sciatic and the tibial, these layers of the pia around the nerve bundle are recognized readily; even in the finest branches, the outer layer of the pia, the perineurium, continues as Henle's sheath.

The Pial Ring

The root slants upward and inward between the two layers of the intima piae and enters the cord through a hole in the inner layer. The margin of this hole is reinforced by a heavy ring of fibers from the intima piae. At the entrance of the relatively large sensory roots, the pial ring is strengthened further by fibrous partitions, dividing it into several smaller rings. Here and there, a small bundle of nerve fibers leaves the main bundle of the root and enters the cord through a small aperture of its own.

The pial ring merits our attention, for it explains the endoneurium. In favorable sections it can be seen plainly that the longitudinal fibers of the endoneurium spring from the pial ring. What appear to be holes in the inner layer of the intima piae are the points where its fibers stream out through the root around the individual nerve fibers to become the longitudinal fibers of the peripheral endoneurium.

The Intramedullary Endoneurium

As shown in Fig. 3, the endoneurium accompanies the nerve fibers of the root for a short distance into the cord; but there is a striking difference in its arrangement outside and inside of the pial ring. Outside, in the root, the strong "longitudinal" fibers run in all directions, binding the nerve fibers together into a bundle. Inside, where the nerve fibers are embedded in the substance of the cord, there seems to be no need of such collective support. Here the binding fibers are reduced to a few delicate filaments that can be traced from one nerve fiber to another. Inside of the cord, support is given rather to the individual nerve fiber by winding strong argyrophil fibers around and around it to form a tubular sheath, very like the tubular sheaths wound around the glomeruli in the ganglia. These winding argyrophil fibers can be traced to the pial ring. The pial ring, then, formed by the inner layer of the intima piae, supplies both kinds of fibers. Facing outward, the pial ring

gives off the longitudinal fibers of the peripheral endoneurium; facing inward, it supplies tubular sheaths to the nerve fibers embedded in the cord.

These argyrophil tubes are seen especially well in thick sections, 20 to 25 microns. After penetrating the cord for a short distance, the bundle of tubes stops abruptly as if chopped off by a knife. The tip of each tube is conical, rounded off as neatly as if turned in a lathe. In many sections, a pale pink axis cylinder from the cord may be seen to enter each conical tip and run inside of the tube formed by the argyrophil web. Counterstaining with azo carmin brings out clearly the position of the axis cylinder inside of the argyrophil web. Motor and sensory roots present exactly the same structure.

The Pial Funnels

At the points where blood vessels from the pia enter the cord, Key and Retzius (1876,[5] page 5 and Table I) describe funnel-shaped extensions of the intima piae (*Piatrichter*) sunk into the cord, forming a loose sheath around the vessel. In silvered sections, these funnels are seen clearly outlined in black. As stated by these authors, here and there a root may enter the cord through such a pial funnel. Most of the roots penetrate the cord accompanied only by the argyrophil endoneurium and the membrane of Schwann.

The Membrane of Schwann

Ranvier (1875,[1] page 1073; 1882,[3] page 1069; 1889,[2] page 805) stated that the membrane of Schwann accompanies the root fibers for a short distance into the marginal glia of the cord and he gives an illustration that is strikingly like Fig. 3. According to Nageotte, this observation has been forgotten. Inside of the cord, the membrane of Schwann and the argyrophil endoneurial sheath have exactly the same distribution.

Technique

We shall give here only a résumé of the technique as modified for the peripheral nerves, referring the reader to the paper in a former number of the Journal (1929) where the methods and the formulas were described in detail.

For Distal Nerves:

1. Large nerves should be split into thin slices to ensure rapid penetration. Fix in Zenker from 3 to 5 hours, no longer. Wash in running water from 3 hours to overnight, as convenient.

2. Embed in paraffin.

3. Stick sections on the slide with Masson's gelatin glue; harden the gelatin in hot formol fumes overnight (*Am. J. Path.*, 1928, **4**, 206; *ibid.*, 1929, **5**, 245).

4. After removal of the paraffin, wash in running water for 5 minutes.

5. Mordant with the Mallory bleach:
   (*a*) 1 per cent tincture of iodin, 3 minutes; rinse in tap water.
   (*b*) 5 per cent hypo, 3 minutes; rinse in tap water.
   (*c*) ¼ per cent potassium permanganate, 5 minutes; rinse in tap water.
   (*d*) 5 per cent oxalic acid, 5 minutes; wash well in running water for 10 minutes.

6. Distilled water; change 3 times within 5 or 10 minutes.

7. Río-Hortega's lithium silver augmented to 10 per cent at 55 to 58° C. for 5 minutes.

8. Quick rinse by pouring distilled water over both sides of the slide.

9. Formol, 1 per cent in tap water, 3 minutes.

10. Rinse with distilled water.

11. Yellow gold chlorid, 1 to 500, at room temperature, 10 minutes.

12. Rinse with distilled water.

13. Oxalic acid, 5 per cent, 10 minutes.

14. Rinse with distilled water.

15. Hypo, 5 per cent, 10 minutes; change as often as it becomes turbid.

16. Wash well in running water to remove the hypo.

Counterstain as desired and mount in balsam. The best counterstains are the reds, such as erythrosin, 1 per cent, or azo carmin, ½ per cent.

The silver solution and the gold solution may be used again and again. Filter the silver solution before use.

For Ganglia:

1. Fix in Bouin's fluid from 1 to 3 days; pass directly to absolute alcohol.
2. Embed in paraffin.

Subsequent steps as for distal nerves except that, in Step 4, Bouin sections should be washed in running water for 20 minutes to remove the picric acid thoroughly; and, in Step 7, the temperature and concentration of the silver bath should be lower. Ten per cent lithium silver at room temperature for 10 minutes or 2 to 3 per cent silver at 40 or 45° C. for 5 minutes give cleaner lines and better detail than the higher temperatures that are necessary for distal nerves.

As noted in the former paper, after Bouin fixation this technique differentiates ectodermic from mesodermic cells. Correspondingly, while all mesodermic cells are invisible, the ectodermic ganglion cells are silver-positive. To obliterate the ganglion cells and secure a pure picture of the collagen framework, as in Fig. 2, we have found several methods effective. The most reliable of them is to repeat the Mallory bleach. After the first Mallory bleach, leave the sections overnight in distilled water, changing it several times. The next day, repeat the bleach and continue from Step 6 as usual.

Ganglia fixed in Zenker not more than 3 hours and stained with silver at 40° C. or under, show good webs and colorless ganglion cells.

For Spinal Nerve Roots:

Fix in Bouin and treat as ganglia. The cord is likely to stain red or black, giving poor contrast with the black web on the roots. Here the double Mallory bleach is useless. A paler ground is secured by a quick rinse with weak ammonia water after the silver bath. The ammonia rinse should not be too long or too strong or the web on the root will be decolorized also. We add 5 drops of ammonia to 100 cc. of distilled water and pour it over the slide for exactly 5 seconds by the watch; then rinse quickly with distilled water and proceed from Step 8 as usual. If the ammonia is to be used, stain the sections at 40 or 45 ° C. Sections stained at room temperature decolorize too easily.

Zenker, Formol and Bouin Fixation:

Formol fixation may be rejected at once. In formol-fixed sections, the web and the finer details of the endoneurium remain invisible.

The universal dependence on formol fixation may be the chief reason why these details were not described long ago.

For distal nerves Zenker is the fixative of choice. For ganglia and roots, Zenker is one of the best fixatives but it is highly selective and somewhat erratic; it sensitizes the various components of the tissue in different degrees and demands some care in selecting the time in the fixative and the temperature and concentration of the silver bath. For ganglia and roots Zenker is a fixative for the expert; Bouin is the fixative for the routine worker, for with routine methods it gives uniform results.

Summary

The endoneurium consists of longitudinal fibers and a closely fitting argyrophil web. The distribution of the web is described together with the silver technique necessary for its demonstration.

This study was commenced and in great part completed in 1927 in the Laboratory of Neurocytology of the Presbyterian Hospital of New York City, at the instance of the Director, Wilder Penfield. The writer takes this occasion to thank both Professor Penfield and Professor William V. Cone for cordial assistance of every kind. That modern master of the histology of the peripheral nerves, Professor Nageotte, of the Collège de France, has been good enough to review and confirm these observations. The writer remains ever indebted to him for sound criticism and advice.

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DESCRIPTION OF PLATES

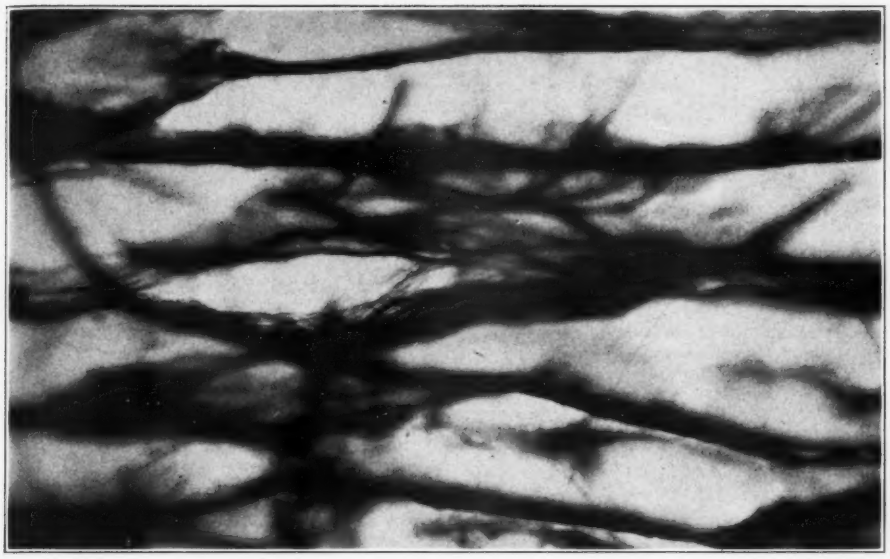
PLATE 93

FIG. 1. Sciatic nerve of cat. Paraffin section. The author's silver technique. The endoneurial fibers (longitudinal fibers and web). The axis cylinders, Schwann cells and myelin sheaths (neurokeratin) are invisible.

FIG. 2. Dorsal root ganglion of cat. Paraffin section. Author's silver technique. A pure picture of the framework of the ganglion; all else invisible. In the upper part of the figure, the web forms fibrous capsules over several (invisible) ganglion cells; over many of the cells it has been cut away.

Center of figure, portion of a glomerulus inside of a fibrous capsule; below this, an entire glomerulus and two fibrous capsules with tops cut off.

Lower part of figure, the endoneurium of the root fibers, showing the longitudinal fibers and the web.



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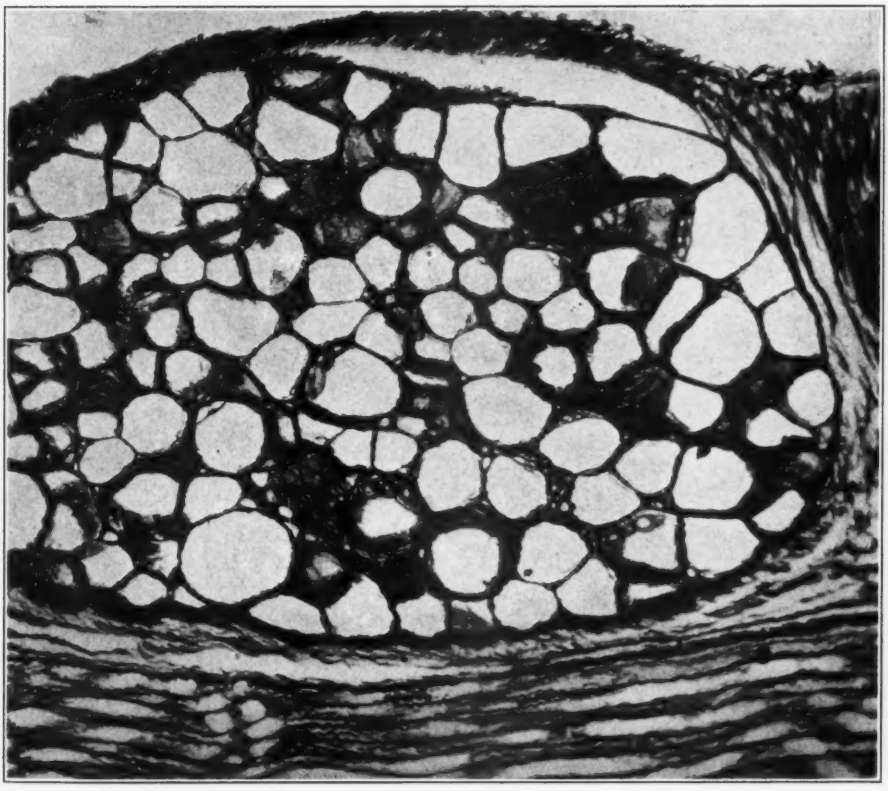

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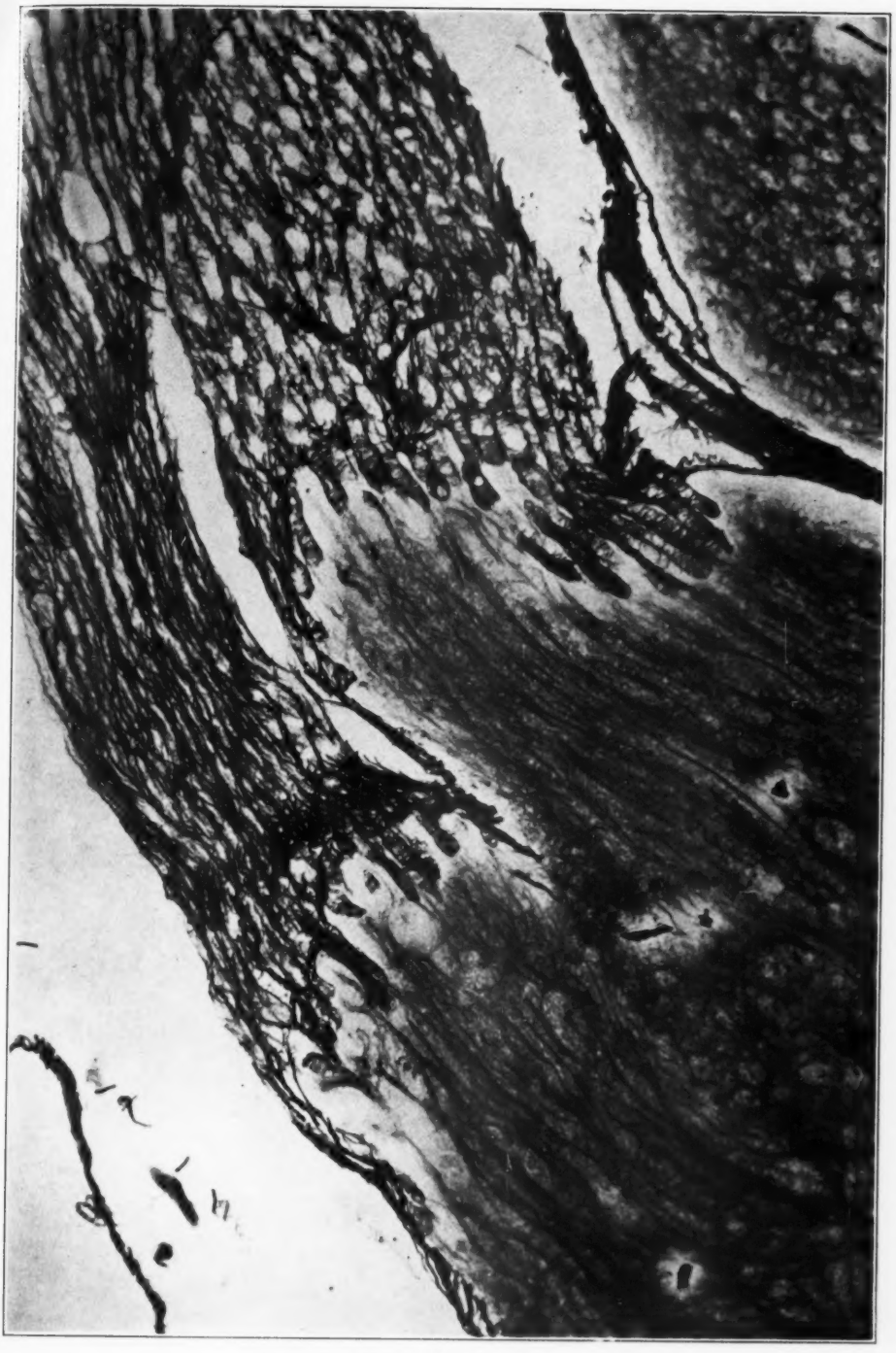
Plate 94

Fig. 3. Cross-section of cord of cat; entrance of sensory root. Paraffin section. Author's silver technique.

Upper left, the endoneurium of the root with prominent longitudinal fibers and web. Center, the pial ring, forming two loops. The longitudinal fibers of the root are continuous with those of the pial ring.

Within the pial ring, the intramedullary endoneurium, accompanying the nerve fiber for a short distance into the cord. Here the longitudinal fibers are few and inconspicuous. The intramedullary endoneurium is seen to consist chiefly of spiral or circular fibers given off by the pial ring: it ends abruptly in a conical tip from which the axis cylinder emerges.



3

Laidlaw Silver Staining of Endoneurial Fibers

A FURTHER MODIFICATION OF DEL RÍO-HORTEGA'S METHOD OF STAINING OLIGODENDROGLIA *

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This modification has been worked out with untiring enthusiasm in our laboratory by the senior technician, Mr. Edward Dockrill. In recognition of this fact it is proposed that the method be called Dockrill's Modification of the silver carbonate method for oligodendroglia.

The method is particularly reliable for staining the oligodendrocytes of the spinal cord, brain stem and cerebral white matter, where other methods are less often successful. It also stains the *sheath* of *Schwann cells* on the peripheral nerves selectively.

FIXATION

Fresh tissue should be fixed in the following solution for 2 hours or up to 1 or 2 days.

Fixative (F. U. P. I.)	Formalin (40 per cent commercial)	20 cc.
	Urea	4 gm.
	Potassium iodide	6 gm.
	Water (doubly distilled)	80 cc.

Cut sections at about 15 microns on the freezing microtome and place in distilled water.

STAINING METHOD †

1. *Wash* in two dishes of distilled water, the first containing 10 drops of ammonia.

2. *Stain* in undiluted silver carbonate ‡ from 1 minute to 1½ hours.

* Received for publication April 28, 1930.

† The numbers correspond with those in Text-Figure 1.

‡ The solution is del Río-Hortega's undiluted ammoniacal silver carbonate made up carefully as follows:

Solution of silver nitrate (Merck) 10 per cent	5 cc.
Solution of sodium carbonate (pure) 5 per cent	20 cc.
Ammonium hydroxide (sufficient to dissolve precipitate).	

The ammonium hydroxide, as indicated above, should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while. Finally, filter and place in a dark bottle, where it will keep for long periods.

3. *Wash* rapidly in 60 per cent alcohol.* The section should be carried through with a small angulated glass rod so as to allow all of it to be washed equally, without wasting time. If the section is wrinkled or folded, the alcohol will produce a patchy result.

4. *Reduce* by passing sections directly into 1 per cent formalin.

5. *Wash* in distilled water.

6. *Tone* by placing sections in gold chloride toning bath † 10 or 15 minutes until they become purple-gray in color.

7. *Fix* in 5 per cent hyposulphite of soda for ½ minute or more until sections are flexible.

8. *Wash* in water.

9. *Dehydrate* in dishes of graded alcohol followed by clearing in carbol-xylol-creosote.‡

10. *Mount* on slide in Canada balsam.

Best results as a rule are obtained by leaving tissues for 5 to 20 hours in the fixative which, for the sake of brevity, is called F. U. P. I. or fupi. But the staining capacity may be revived in an overfixed subject by placing sections in 4 per cent urea overnight and then passing them directly into the silver bath for an hour or less. If the subject has been fixed by preliminary carotid injection, it is better to try for oligodendroglia within 2 hours or less after the block has been placed in fixative.

To stain oligodendroglia in sections of the *spinal cord, optic nerve* and *retina,* place small fresh pieces in the above fixative to which has been added 4 gm. of chloral hydrate. Our best results were obtained by leaving fresh blocks of tissue from 2 to 4 days in this fixative. Cut sections and proceed as above.

Creditable staining of oligodendrocytes has been obtained from old formol material in the following manner: Blocks were cut and placed in 15 per cent ammonia water for 24 hours. They were then washed in running tap water overnight and placed in the F. U. P. I. fixative for a week. Sections were then cut and left in 4 per cent urea overnight and stained as before. This applies to brain tissue. We have had no marked success with old formalin-fixed spinal cords.

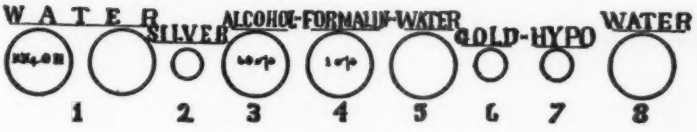
* Commercial alcohol (95 per cent) usually contains some impurity. When diluted there appears a slight opalescence. This is prejudicial to the success of the staining. For this reason we have always used absolute alcohol in preparing the 60 per cent.

† Toning bath: Gold chloride (yellow) ..... 1 gm.
Distilled water ..... 500 cc.

‡ Carbolic acid 10 cc., creosote 10 cc., xylol 80 cc.

Results

More complete staining of the oligodendrocytes in the white matter of the brain and spinal cord may be obtained by this modification of del Río-Hortega's method (Figs. 1 and 2), although the results in the gray matter are less delicate and satisfactory than by the original method of that author [1] or the modification for microglia and oligodendroglia by Penfield.[1]



Text-Figure 1

Order of staining procedure from left to right. Numbers correspond with the text.

The recently described method of del Río-Hortega,[2] which is a modification of Golgi's chrome silver method, occasionally gives results which for complete staining of the oligodendrocyte expansions are unequalled. But the results are so unequal and the staining so powdery as to make it so far of little use for routine work.

The method described here has been found particularly useful by Dr. Cone in staining the ologodendroglia of the retina, nerve head and optic nerves. Microglia is also stained with varying success by this method.

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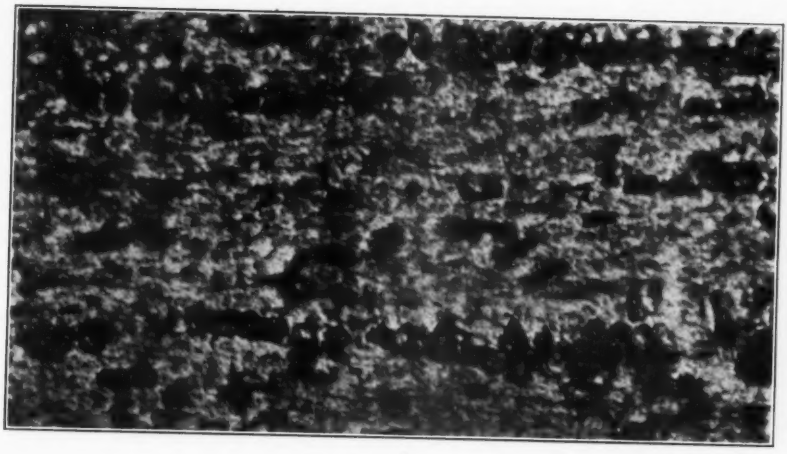
DESCRIPTION OF PLATE

PLATE 95

FIG. 1. Rows of oligodendrocytes in the cerebral white matter of a dog; normal.

FIG. 2. Higher magnification of oligodendrocytes from the white matter of the same animal.



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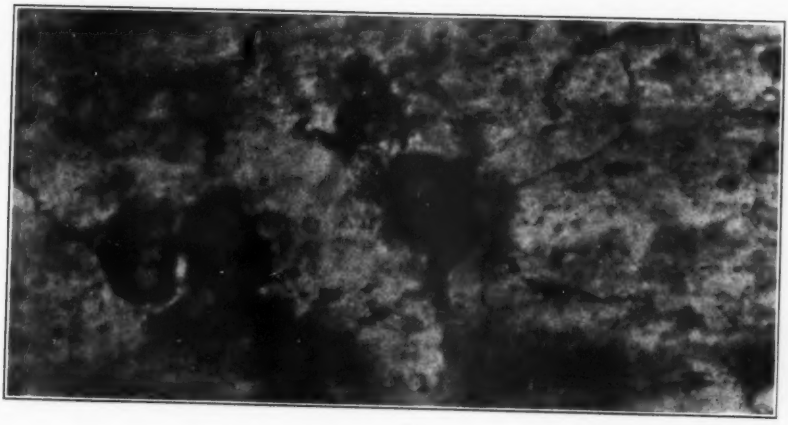

2

RESULTS FOLLOWING INTRARENAL ARTERIAL TUBERCULIN INJECTIONS IN NORMAL AND TUBERCULOUS MONKEYS, GOATS AND SWINE *

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In a previous investigation [1] it was shown that injection of the specific protein of tuberculin into the renal artery of tuberculous swine resulted in acute inflammation involving glomeruli and interstitial tissue. The fact that similar injection into normal swine failed to cause an inflammatory reaction, indicated that the effect in the tuberculous animal was a true tuberculin reaction.

The experiments were not reported as suggesting a relationship between tuberculosis and spontaneous nephritis in man, but simply as an example of one possible effect, in a chronically infected and therefore hypersensitive animal, of the substance to which the animal is hypersensitive. The results seemed of possible significance for the general field of chronic infection and allergy.

The dosage used to produce the effects recorded, however, while insufficient to produce effects in normal control animals, was nevertheless much larger than any amount that could be liberated spontaneously from a focus of disease in any infected animal. It therefore seemed necessary before application could be made to spontaneous nephritis, to determine if much smaller amounts could produce lesions like those of naturally occurring disease. It seemed desirable, also, to determine if the condition noted for swine held for other animals.

Accordingly a study was made of the effect of smaller amounts of tuberculin protein in monkeys and goats. A number of swine were again studied. The purified tuberculin protein of Florence Seibert, prepared by ultrafiltration,[2] was used, and in amounts varying from 5 to 35 milligrams. The material for injection was secured in the form of a fine flocculent suspension by diluting the pure solution of tuberculin protein with an anticoagulating solution of salt and

* Aided by a grant from the Medical Research Committee of the National Tuberculosis Association.

Received for publication April 17, 1930.

sodium citrate (respectively 0.5 and 1 per cent), acidifying faintly with hydrochloric acid, barely bringing to the boiling point and neutralizing. This treatment does not injure tuberculin. As noted in the previous article, it is necessary to inject particulate matter for the success of this experiment, in order to localize the effects of the substance. Injecting clear, fluid tuberculin elicites only the general effects of this substance. Injection was made in exactly the same manner as in the first investigation. The effect of the injections was determined by biopsy two to eight days later, and by the final picture at autopsy.

All animals were subjected to the tuberculin skin test before the injections were made, and distinct differences were noted in the three groups of animals. All animals were infected with the human type of tubercle bacillus except in the case of three goats. Goats proved highly susceptible to bovine infection, the three animals injected succumbing in four to six weeks with widely disseminated tuberculosis, from a subcutaneous injection of 2 milligrams of bacilli (see protocols for Goats 1, 2 and 3 below).

All infected animals were skin sensitive in some degree to tuberculin. In the monkeys the zone of cutaneous reaction was wide in extent but extremely soft and pale. The red injection and the induration characteristic of the reaction in the guinea pig and man were absent. The goats gave strong reactions, with a redness and firm area of inflammatory edema surpassing that which we have seen in any other animal. The swine gave red zones of inflammatory induration of feeble intensity.

The monkeys (Macacus rhesus) averaged about five pounds in weight, the goats thirty pounds, and the pigs forty (at the outset). The results of the experiment are summarized in the following protocols.

Monkeys

Monkey 1. Tuberculous

Feb. 25, 1928: 0.1 mg. of H 37 tubercle bacilli injected intraperitoneally.

April 15, 1928: Tuberculin test weakly positive.

May 4, 1928: Right kidney injected with approximately 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

May 11, 1928: Biopsy of right kidney. Specimen showed no changes of any significance.

June 1, 1928: Left kidney similarly injected.

June 4, 1928: Biopsy of left kidney. Specimen showed localized regions of degeneration from vascular injury, and a more diffuse involvement characterized by the presence of many dense hyaline casts. Many of these contained polymorphonuclear leucocytes. No other inflammatory changes were seen.

July 12, 1928: Monkey died. Autopsy: generalized abdominal lymphatic tuberculosis. Sections from the right kidney (injected May 4) showed no changes except a few small zones of lymphocytic infiltration. The left (injected June 1) was similar, except that, in addition, a few healed infarcts were present.

Monkey 2. Tuberculous

Feb. 25, 1928: 0.1 mg. of H 37 injected intraperitoneally.

April 15, 1928: Tuberculin test weakly positive.

April 27, 1928: Right kidney injected with approximately 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

May 4, 1928: Biopsy of right kidney. The only changes from the normal were a slight proteinuria, and a few old scars infiltrated with lymphocytes, which presumably dated from an unknown, much older injury.

May 21, 1928: Left kidney similarly injected.

May 29, 1928: Biopsy of left kidney. Some cortical necrosis due to vascular injury was found, and casts were numerous throughout the section. Practically no inflammatory changes were seen.

July 13, 1928: Monkey died. Autopsy: generalized abdominal and lymph node tuberculosis. In the right kidney no changes of significance were seen. The left kidney was normal except for cortical regions of healed infarction.

Monkey 3. Tuberculous

Feb. 25, 1928: 0.1 mg. of H 37 injected intraperitoneally.

April 15, 1928: Tuberculin test weakly positive.

May 14, 1928: Right kidney perfused with approximately 5 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

May 23, 1928: Biopsy of right kidney. No changes of significance found.

July 17, 1928: Monkey died. Autopsy: generalized abdominal lymph node tuberculosis. Right kidney: no changes of significance. Left kidney: normal.

Monkey 4. Normal

May 15, 1928: Tuberculin test negative.

May 25, 1928: Right kidney perfused with approximately 5 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

June 5, 1928: Biopsy of right kidney. No changes of significance found. After the biopsy this monkey was transferred to another experiment. A second operation was not performed, as we were interested at the time only in the effect of tuberculin protein on the absolutely normal animal.

Monkey 5. Normal

May 15, 1928: Tuberculin test negative.

June 4, 1928: Right kidney perfused with 5 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

June 11, 1928: Biopsy of right kidney. No changes of significance found. After the biopsy this monkey also was transferred to another experiment.

A number of determinations of blood chemistry were made on the monkeys of this series by Dr. Lucy Finner, but at no time were values outside of the normal range encountered.

Goats

Goats 1 and 2. Tuberculous

July 23, 1928: 2 mg. of bovine type tubercle bacilli (B 1698) injected in left groin, and 2 mg. of human type bacilli (H 37) injected in right groin. In the succeeding weeks the lymph glands on the side of the bovine infection increased greatly in size, while those on the side infected with human bacilli increased only moderately. Neither of these goats lived long enough to be used for the experiment. Goat 1 died on Sept. 3, 1928, and Goat 2 on Sept. 7, 1928, each with very extensive pulmonary miliary tuberculosis.

Goat 3. Tuberculous

July 23, 1928: 2 mg. each of bovine type tubercle bacilli (B 1698) and human type bacilli (H 37) injected into opposite groins. In the succeeding weeks the glands on the side of the bovine infection increased greatly in size, while those on the side infected with human type bacilli increased only moderately.

Sept. 1, 1928: Tuberculin test strongly positive.

Sept. 10, 1928: Right kidney injected with a suspension of 10 mg. of coagulated tuberculin protein in salt-citrate solution.

Sept. 12, 1928: Biopsy of right kidney. Marked degeneration of the tubular epithelium with some interstitial infiltration with polymorphonuclear leucocytes was found. The glomeruli were unchanged except for the presence of a good many leucocytes. Many of the tubules contained hyaline casts.

Sept. 17, 1928: The left kidney was similarly perfused with a suspension containing 20 mg. of coagulated tuberculin protein. Marked shivering developed in the animal during the injection.

Sept. 19, 1928: Goat died. Autopsy revealed very extensive pulmonary miliary tuberculosis. Sections of the right kidney showed extensive interstitial lymphocytic infiltration, in place of the polymorphonuclear leucocytic reaction seen in this kidney on September 12. Much regeneration of the epithelium of this kidney was found. Many hyaline and leucocytic casts were present in the tubules. The glomeruli appeared unchanged. The left kidney showed changes similar to those seen in the right kidney September 12, except that the inflammatory features were less marked. Much degeneration of the tubular epithelium was seen.

Goat 4. Tuberculous

Sept. 10, 1928: Tuberculin test negative.

Sept. 20, 1928: 3 mg. of H 37 injected in left groin. In succeeding weeks marked enlargement of the regional inguinal glands developed.

Oct. 25, 1928: Tuberculin test strongly positive.

Oct. 31, 1928: Right kidney perfused with 10 mg. of coagulated tuberculin protein in salt-citrate solution. A mild general reaction with shivering occurred.

Nov. 2, 1928: Biopsy of right kidney. Specimen showed vascular injury and anemic necrosis. No inflammatory changes were present.

Nov. 26, 1928: Left kidney injected similarly.

Nov. 28, 1928: Biopsy of left kidney. Specimen showed no changes except marked proteinuria. No appreciable tubular degeneration, no casts, no inflammatory changes.

Jan. 9, 1929: Tuberculin test much weaker than on October 25.

Jan. 19, 1929: 4 mg. of H 37 injected in right groin.

Mar. 1, 1929: Tuberculin test strongly positive.

Mar. 13, 1929: Right kidney exposed for second injection, but found completely atrophied (complete infarction from vascular injury).

July 15, 1929: 1 mg. of bovine tubercle bacilli injected in right axilla.

Aug. 29, 1929: Left kidney exposed, biopsy specimen taken and 10 mg. of tuberculin protein injected into renal artery. The biopsy specimen resembled that seen on Nov. 28, 1928.

Oct. 11, 1929: Goat killed. Right kidney could not be found. Left kidney showed little change grossly. Microscopically a number of regions of periglomerular lymphocytic infiltration were seen, and a few small fibrous scars. Fusion of tuft and capsule was seen in a few glomeruli. Casts were present in many of the tubules. The lymph nodes regional to the points of injection were the seat of a mild fibrocaseous tuberculosis. There was no disseminated tuberculosis.

Goat 5. Tuberculous

Sept. 10, 1928: Tuberculin test negative.

Sept. 20, 1928: 3 mg. of H 37 injected in left groin.

Oct. 25, 1928: Tuberculin test strongly positive.

Nov. 5, 1928: Left kidney injected with 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

Nov. 7, 1928: Biopsy of left kidney. Section revealed marked proteinuria, and no inflammatory changes.

Dec. 3, 1928: Right kidney injected similarly.

Dec. 5, 1928: Biopsy of right kidney. Section revealed a marked proteinuria and many dense hyaline casts in the tubules. Mitotic figures were numerous in the tubular epithelium. A few minute regions of periglomerular lymphocytic infiltration were seen, and minute collections of polymorphonuclear leucocytes were occasionally found in the glomerular capillaries.

Dec. 10, 1928: Goat very sick.

Dec. 11, 1928: Goat died with generalized peritonitis. Sections showed both kidneys practically normal.

Goat 6. Tuberculous

Sept. 10, 1928: Tuberculin test negative.

Sept. 20, 1928: 3 mg. of H 37 injected in left groin.

Oct. 25, 1928: Tuberculin test strongly positive.

Nov. 12, 1928: Right kidney injected with approximately 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

Nov. 14, 1928: Biopsy of right kidney. Large numbers of dense hyaline casts were found in the renal tubules. The tubular epithelium showed only mild degeneration. Focal periglomerular lymphocytic infiltration was frequently found. There was no necrosis or vascular damage. There was some perirenal suppuration.

Jan. 9, 1929: Tuberculin test weakly positive.

Jan. 17, 1929: Left kidney injected with 35 mg. of coagulated tuberculin protein.

Jan. 19, 1929: Biopsy of left kidney. Sections revealed proteinuria, but no casts and no inflammatory changes. 4 mg. of H 37 injected in right groin.

Feb. 20, 1929: Tuberculin test positive.

Feb. 27, 1929: Right kidney (first injected on Nov. 12, 1928) exposed and biopsy taken. 25 mg. of coagulated tuberculin protein injected. Biopsy section revealed no changes except a few minute spots of slight lymphocytic infiltration (Fig. 1).

Mar. 1, 1929: Goat killed. In the right kidney (injected February 27 and previously on November 12) an enormous number of hyaline and waxy casts were seen (Fig. 2). Very little degeneration of the tubular epithelium was apparent. No vascular damage or necrosis was seen, and inflammatory changes were absent. The left kidney (injected January 17) was practically normal.

Goat 7. Normal

Sept. 10, 1928: Tuberculin test negative.

Oct. 10, 1928: Left kidney injected with 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

Oct. 12, 1928: Biopsy of left kidney. Sections revealed almost no changes. A very few casts were found.

Oct. 22, 1928: Right kidney similarly injected.

Oct. 24, 1928: Biopsy of right kidney. Sections revealed no changes except proteinuria.

Feb. 13, 1929: Right kidney again injected, with 30 mg. of coagulated protein.

Feb. 15, 1929: Right kidney exposed for biopsy and found completely infarcted. Renal artery thrombosed. Kidney removed.

Oct. 19, 1929: Goat killed. Left kidney (injected Oct. 10, 1928) essentially normal.

Goat 8. Normal

Sept. 10, 1928: Tuberculin test negative.

Oct. 17, 1928: Right kidney injected with 10 mg. of coagulated tuberculin protein. Injection attended with unusual amount of trauma.

Oct. 19, 1928: Biopsy of right kidney. Sections for the most part quite normal. One infarcted region full of casts found; tubules in this region swollen and vacuolated; thrombosed artery not found in section.

Nov. 19, 1928: Left kidney injected in similar manner.

Nov. 21, 1928: Biopsy of left kidney. Section revealed no changes except proteinuria.

Dec. 6, 1928: Goat died, with ascites and bilateral hydrothorax. Both kidneys were found completely infarcted from thrombosis of the renal arteries.

SWINE

Pig 1. Tuberculous

May 1, 1929: Infected in groin with 2 mg. of human type tubercle bacilli.

June 4, 1929: Tuberculin test positive, moderate degree.

June 10, 1929: Right kidney injected with 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

June 12, 1929: Biopsy of right kidney. Moderate changes were seen with hematoxylin and eosin stains. The tubular epithelium was swollen. Many hyaline and a few leucocytic casts were seen in the tubules. A few patches of interstitial lymphocytic infiltration

were seen. No glomerular changes could be found. Sudan III stains revealed profound fatty degeneration of the tubules.

July 11, 1929: Left kidney injected in similar manner to right on June 10. A gasping respiration developed and the pig died just as the injection was finished. Autopsy: fibrocaseous tuberculosis of the inguinal lymph nodes regional to the site of infection with tubercle bacilli. No other tuberculosis. The right kidney, injected June 10, appeared normal except for a few patches of interstitial lymphocytic infiltration and a slightly increased cellularity of the glomeruli. The left kidney, injected just before death, was normal.

Pig 2. Tuberculous

May 1, 1929: Infected in groin with 2 mg. of human type tubercle bacilli.

June 4, 1929: Tuberculin test positive, moderate degree.

June 12, 1929: Right kidney injected with 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

June 14, 1929: Biopsy of right kidney. Small regions of infarction from vascular occlusion were found. Outside of the infarcted regions a good many hyaline and a few leucocytic casts were found in the tubules. The glomeruli appeared normal. A few patches of interstitial lymphocytic infiltration were seen. There was some perirenal suppuration, presumably from infection at the time of operation. Sudan III stains revealed a profound fatty degeneration of the tubular epithelium.

June 19, 1929: Left kidney injected in similar manner to right on June 12. As in the case of Pig 1 the animal collapsed and died just as the injection was finished. Autopsy: fibrocaseous tuberculosis of the inguinal lymph glands regional to the site of infection with tubercle bacilli. No other tuberculosis. The right kidney (Fig. 3), injected June 12, was the seat of a marked interstitial nephritis, with intertubular edema, a profound interstitial lymphocytic infiltration, and many casts formed from polymorphonuclear leucocytes in the tubules. Leucocytes were numerous in the glomeruli, which were otherwise normal for the most part, but occasionally showed a slight proliferative change. The left kidney, injected just before death, showed no change except some protein precipitate in the tubules.

Pig 3. Normal

May 22, 1928: Right kidney injected with 20 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

May 24, 1928: Biopsy of right kidney. Little change seen. One area of tubular necrosis from arterial obstruction was seen. No signs of inflammation were found. Sudan III stains showed no fatty degeneration.

June 4, 1929: Tuberculin test negative.

June 5, 1929: Left kidney injected in similar manner to right on May 22.

June 7, 1929: Operation for biopsy of left kidney. Kidney was found completely infarcted and was removed.

Aug. 25, 1929: Pig killed. Sections showed the right kidney to be practically normal. A few cortical scars from old infarction were seen, and an occasional fusion of glomerular tuft and capsule was noted.

Pig 4. Normal at First. Later Tuberculous

May 24, 1928: Right kidney injected with 20 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

May 27, 1928: Biopsy of right kidney. Sections showed almost no change. A very few leucocytic casts were found.

June 4, 1928: Tuberculin test negative.

June 7, 1928: Left kidney injected in similar manner to right on May 24.

June 9, 1928: Biopsy of left kidney. Extensive gross infarction found. Sections showed extensive thrombosis, infarction and a good many polymorphonuclear leucocytes in the infarcted areas. Sudan III sections showed fatty degeneration limited to the infarcted areas.

July 15, 1928: Pig injected in groin with 2 mg. of bovine type tubercle bacilli. Marked enlargement of the regional lymph nodes developed in the following weeks and then subsided.

Aug. 24, 1928: Right kidney injected with 5 mg. of coagulated tuberculin protein in usual manner.

Oct. 11, 1928: Pig killed. Autopsy: fibrocaseous tuberculosis of lymph nodes regional to site of infection with tubercle bacilli. No disseminated tuberculosis. Right kidney smaller than left. Sections of each kidney revealed no significant microscopic changes.

Summary of Results in Monkeys

Skin sensitiveness to tuberculin in the tuberculous monkeys of this experiment, infected intraperitoneally with tuberculosis, was low. Likewise only mild injury of the kidneys of these tuberculous monkeys could be produced by injection of a suspension of tuberculin protein containing particles of a sufficient size to obstruct glomerular capillaries. Aside from the occasional abrupt necrosis resulting from vascular thrombosis subsequent to injection of the artery, only mild degenerative changes with the production of a few casts, were seen in the tuberculous monkeys of this series, and inconstantly in these. In no case were the inflammatory changes characteristic of the tuberculin skin reaction in sensitive animals found. No changes were produced in normal animals.

Summary of Results in Goats

Goats infected subcutaneously with bovine type tubercle bacilli rapidly succumbed with extensive pulmonary miliary tuberculosis. One goat out of three so infected, which lived long enough for further experiment, proved strongly skin sensitive to tuberculin and developed a moderate acute interstitial nephritis, with pronounced tubular degenerative changes, on arterial injection of the kidneys with tuberculin protein. Three goats injected subcutaneously with human type tubercle bacilli gave strongly positive tuberculin skin tests five weeks later. Renal arterial injections of the kidneys of these animals resulted in slight tubular degenerative changes in one, acute degenerative changes on the second of two injections, with slight inflammation, in a second, and acute tubular degeneration with many hyaline and waxy casts in a third. A second injection of tuberculin protein into the same kidney of the latter animal, three months later, led to the production of an enormous number of dense hyaline casts with no appreciable inflammation and little apparent tubular degeneration. Injection of tuberculin into normal control goats led to no changes, except those due to accidental vascular injury.

Summary of Results in Swine

Tuberculous swine proved only moderately skin sensitive to tuberculin. Injection of tuberculin protein into the renal artery in tuberculous swine, however, produced much more marked changes than occurred from similar treatment of either monkeys or goats. The lesions produced with the dosage of tuberculin used (10 mg.) were not as severe as those previously reported in swine following similar injection of large quantities of tuberculin protein (30–100 mg.). In particular the glomerular lesions noted in the former investigation were not repeated. On the other hand quite similar interstitial changes occurred. As in the former study, recovery from the lesions produced, with restoration practically to normal, occurred in the course of a few weeks after the injury. In more detail the results in swine were as follows: Renal arterial injection of suspended coagulated tuberculin protein in two tuberculous swine caused profound fatty degenerative changes in the tubular epithelium with hyaline and leucocytic casts. In one of these there was marked and in the other moderate interstitial infiltration with cells of inflammation. A second renal arterial injection, in the opposite kidney, led to abrupt exitus on the operating table in each of these animals (compare with asthma and shock in tuberculous animals of first investigation following injection of tuberculin protein). Two normal control pigs, similarly injected, showed no changes except lesions obviously the result of vascular thrombosis or embolism, a type of accident which occurred occasionally throughout the whole series of animals in this study.

General Summary

Distinct renal allergic responses were secured on the injection of tuberculin protein into the renal arteries of tuberculous monkeys, goats and swine. The allergic nature of the response was established by the fact that similar injection into normal controls did not cause injury (except such as occurred from vascular occlusion). In the monkeys the lesion produced was purely degenerative, in the goats chiefly degenerative but occasionally inflammatory, and in the swine degenerative and of a more inflammatory character than in the goats. The inflammation in the goats and swine took the

form of an interstitial nephritis. The glomerular changes observed in a former investigation in which larger dosage of tuberculin protein was used, were not produced. The intensity of renal tuberculin reaction did not parallel the intensity of cutaneous reaction in this series of animals.

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DESCRIPTION OF PLATE

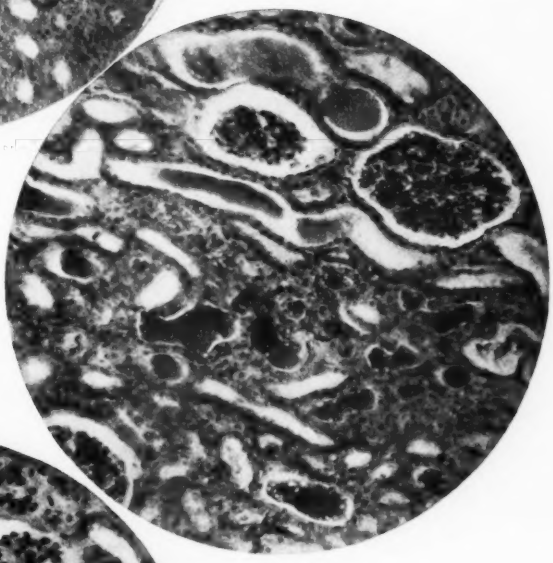
PLATE 96

FIG. 1. Right kidney of tuberculous Goat 6 on Feb. 27, 1929. Essentially normal three and one-half months after renal tuberculin reaction (Nov. 12, 1928). × 125.

FIG. 2. Right kidney of tuberculous Goat 6 on Mar. 1, 1929, two days after injection of tuberculin protein into right renal artery. (Fig. 1 shows the appearance of this kidney before the injection, as revealed by biopsy.) × 135.

FIG. 3. Right kidney of tuberculous Pig 2 seven days after injection of tuberculin protein into right renal artery. Biopsy five days previously had revealed a more acute reaction. × 240.

1



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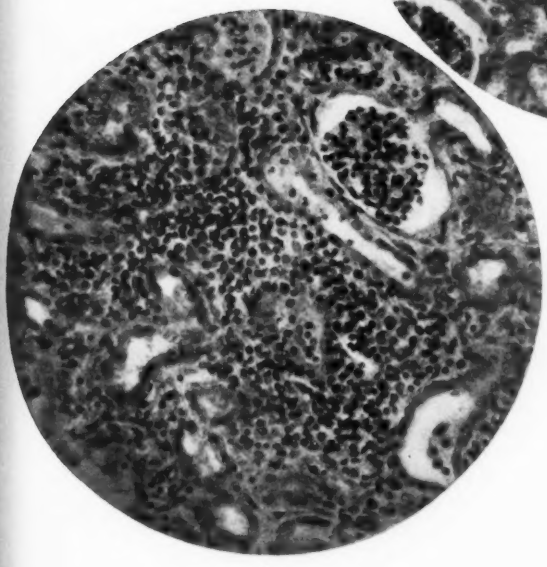

3

Long, Huggins and Vorwald Intrarenal Arterial Tuberculin Injections

MARKED DILATATION OF THE LEFT AURICLE OF THE HEART *

Report of a Case

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Marked dilatation of the left auricle of the heart is a relatively rare finding at the autopsy table. This is the report of a case coming to autopsy at the Bellevue Hospital.

Review of Literature

The original clinical and pathological descriptions of extreme left auricle dilatation were contributed by Owen and Fenton [1] in 1901. Their patient presented symptoms of right pleural effusion for which a thoracentesis was performed and pure blood obtained. At autopsy the left auricle was markedly dilated and contained 30 ounces of blood, as compared with the normal content of 2 to 4 ounces. Shaw [2] reports a heart whose left auricle held 30 ounces. Emanuel [3] reports a heart whose left and right auricles held 40 ounces and 20 ounces respectively. In East's patient [4] the left auricle held 1½ pints (23 ounces) of blood and the auricular wall was so thinned that no muscle could be detected.

Schott [5] describes the pathogenesis of left auricular dilatation and demonstrates by orthodiagrams the progressive enlargement of the auricle.

Bordet [6] conducted X-ray studies in a large series of cases showing mitral stenosis and found the left auricle dilated to the right of the right auricle in 5 per cent of the cases.

Bedford [7] states that early diagnosis of left auricular dilatation beyond the right auricle is possible only by X-ray, but that later suggestive signs and symptoms of its presence may appear.

Bach and Keith [8] describe a patient with active rheumatic fever who came to autopsy and presented a dilated left auricle which widened the interbronchial angle and compressed the left bronchus.

* Read before the New York Pathological Society, November 21, 1929.
Received for publication December 10, 1929.

Case Report

Clinical History: A.D. (B. H., chronic cardiac valvular disease 2518 — 2nd Div.), an Italian male, 23 years of age, entered the hospital by ambulance on October 10, 1929, in a stuporous, semi-moribund state of acute cardiac decompensation.

Present Illness: No history was obtained from the patient. His brother stated that the patient had been ill for a long time with heart trouble and worked irregularly. The brother found the patient on the floor of his home and believed him to be almost dead.

Physical Examination: A young, fairly well developed, poorly nourished Italian male, extremely dyspneic and orthopneic with cyanosis of the lips and finger-tips, was lying propped up in bed. The respirations were shallow and rapid (24), temperature 100° F, and pulse 112. The patient was unable to talk and had a right facial weakness. There were petechial hemorrhages in the right conjunctiva and over the skin of the chest and arms. The mucous membranes were pale. The chest was thin-walled and the expansions were equal. The breath sounds over the anterior portions of the chest were harsh and loud with sibilant and sonorous râles predominating. Posteriorly, the breath sounds were harsh, vesicular, with numerous moist râles at both bases.

The apex beat of the heart was visible and palpable in the fifth and sixth intercostal spaces from the nipple to the anterior axillary lines. The heart was enlarged to the right as its border of dullness was 5.0, 5.0, 8.5 cm. to the right of the midsternal line in the second, third and fourth intercostal spaces respectively. The rhythm was totally irregular. The apex beat was 132, and the radial pulse 112 per minute, a pulse deficit of 20. There was a marked systolic thrill at the apex. A short presystolic murmur followed by a prolonged blowing murmur, maximum at the apex, and replacing the first sound was heard over the precordia. The pulmonary second sound was greater than the aortic second sound. The pulses were equal, small and totally irregular.

The liver edge was not felt. The right upper and lower extremities lay limply at the patient's side completely paralyzed. Deep tendon reflexes were present on both sides. No Babinski.

Laboratory Findings: The urine was yellow, acid, specific gravity 1.018, with a slight trace of albumin, but no sugar.

The blood non-protein nitrogen was 45, and the sugar 75 mg. per 100 cc.

The Wasserman was negative. Cytological studies of the blood were not conducted.

Progress Notes: The patient became more cyanosed, temperature progressed to 103° F and he died thirty-two hours after entering the hospital.

Clinical Diagnoses: Chronic cardiac valvular disease (rheumatic) with auricular fibrillation, embolism of the left internal capsule, and subacute bacterial endocarditis (?).

Autopsy Report

The autopsy was performed on October 11, 1929 (Accession Number 14736).

The body weighs 115 pounds and measures 175 cm. in length. The chest and arms contain numerous small petechial hemorrhages. There is no edema present.

Pericardium: The sac is markedly distended and extends 8 cm. to the right and 7 cm. to the left of the midsternal line at the level of the diaphragm. The cavity contains 150 cc. of straw-colored fluid. The pericardial surfaces are smooth, glistening and contain no adhesions.

Heart: Weight 540 gm. The left auricle is markedly dilated and its right border extends 8 cm. to the right of the midclavicular line in the region of the fourth rib. The lung tissue is compressed. The left border of the left auricle lies in the arch of the aorta. The left auricle has a capacity of 593 cc. of fluid (after fixation). The right auricle is slightly dilated. The auricular appendages are natural. Section through the left auricle shows an increase in thickness of the wall. The auricular endocardium contains a thin mural thrombus covering most of the surface. The opened left auricle (circumference) measures 25 cm. laterally and 15 cm. (circumference) above the mitral valve. The ventricular myocardium is apparently not hypertrophied.

The mitral valve is stenosed to a slit-like opening whose base is calcified and whose opening is 2.2 cm. in length and will not admit the smallest digit. The leaflets of the aortic valve are adherent, slightly thickened and the orifice is stenosed. The cardiac measure-

ments are: tricuspid valve 12.5, pulmonary valve 6.8, mitral valve 4.0, aortic valve 6.2, left ventricle 1.2, and right ventricle 0.3 cm.

The other organs are not remarkable.

Anatomical Diagnoses: Stenosis of mitral and aortic valves, hypertrophy and marked dilatation of left auricle, mural thrombus left auricle.

Microscopic Examination

Heart: The ventricular musculature is normal. The nuclei of the auricular musculature are large and the fibers are hypertrophied. The muscle fibers immediately below the endocardium are necrotic and have lost their nuclei. The auricular myocardium is infiltrated with lymphocytes.

Lungs: Many pigment-containing endothelial leucocytes are present.

Liver: Pigment present in the cells about the central veins.

The other organs are not remarkable.

Discussion

The auricular dilatation, as described here, is associated with mitral stenosis of rheumatic origin. Many cases show more marked mitral stenosis with little or no auricular dilatation. Emanuel [3] believes that the auricular dilatation is due to auricular myocardial damage. To prove this point he describes a heart in St. Bartholomew's Hospital Museum,[9] whose left auricle is markedly dilated yet the mitral valve admits three fingers. A heart in the University College Hospital[10] is similar and the mitral valve is only slightly thickened. Bach and Keith [8] present a heart with marked left auricular dilatation whose mitral valve admits three fingers. This case, however, as most cases cited in literature, presents a pericarditis with the valvular lesions. This reaction is symbolic of a pancarditis which includes the myocardium of the left auricle. Increased intra-auricular pressure through an incompetent mitral valve plus a damaged myocardium will probably account for marked left auricular dilatation.

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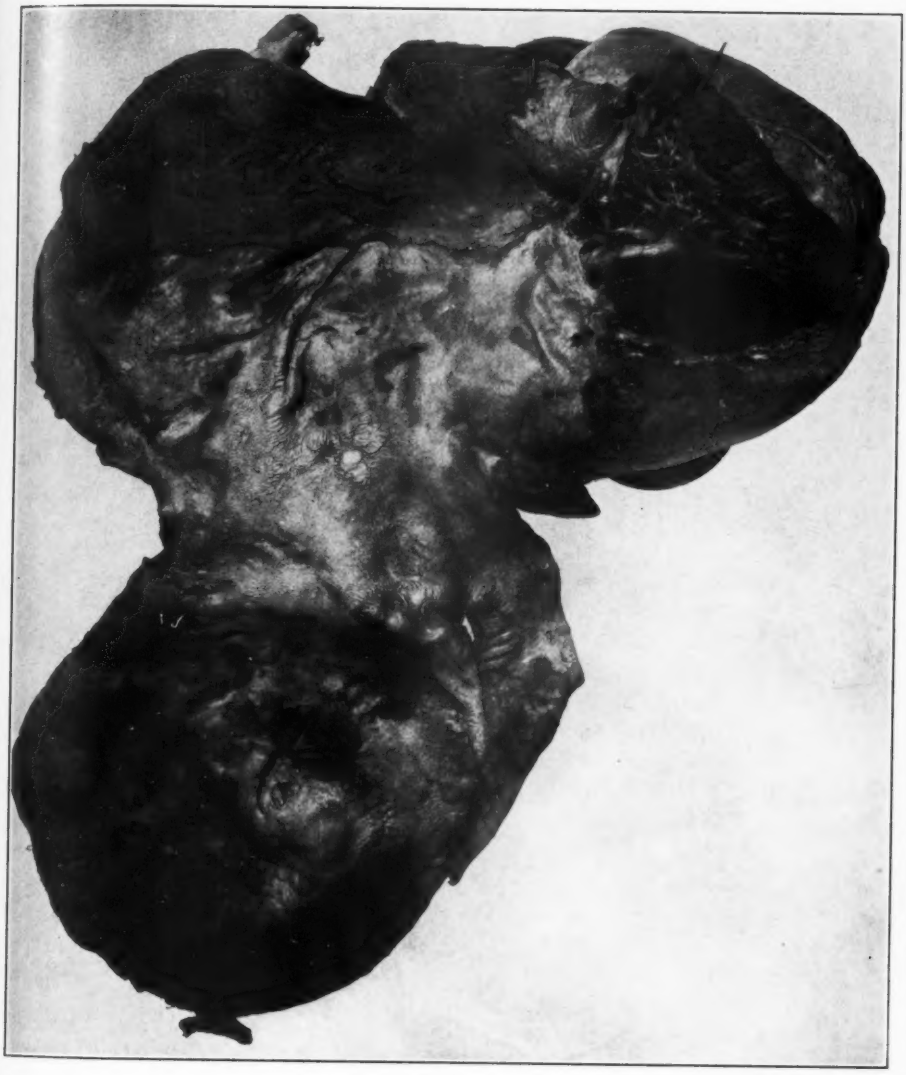
DESCRIPTION OF PLATE

PLATE 97

FIG. 1. Dilated left auricle contains a thin mural thrombus. The mitral valve is stenosed and calcified.



Burkhardt Dilatation of Left Auricle of Heart

HISTOLOGICAL STUDIES ON THE BRAIN OF A CRANIOPAGUS *

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INTRODUCTION

Craniopagus is one of the most infrequently occurring symmetrical malformations. In the literature only twenty-two cases have so far been reported, the first one dating back to a report by Sebastian Münster [1] in 1495.

Three types of craniopagus are generally recognized according to the part of the skull that is involved: first, the frontal; second, the occipital; third, the parietal regions. The first type occurs most infrequently, and up until now only three cases have been reported (one each by Münster,[1] Baer [2] and Warschauer).[3] The one reported by Münster is the most remarkable in that the children lived for ten years. They were six years of age when they first came under the observation of Münster in 1501. Baer's report (1845) consists of a short description of a museum specimen with a statement that "the brains communicated through an opening in the frontal bones." Warschauer's description was published in 1909. In his case the faces of the children were quite well developed up to the eyebrows. The left sides of both faces were somewhat smaller than the right. There was no further study of the brains, the reason being, as stated by him, that the tissues were "decomposed." The general structure of the two brains, as far as could be seen, was asymmetrical, and there was no definite dural fold between the two. The falx cerebri seemed to be pushed over to the left, making the space containing the right cerebral hemisphere about twice as large as the left. The brains were "probably separate."

The number of parietal craniopagi reported is fourteen. Twelve of these were collected by Ahlfeld [4] in his monograph in 1882. Two cases were added recently (Kissinger [5] in 1908, and Kafka [6] in 1920). The case which I wish to present also belongs to this group, making a total of fifteen.

* Received for publication March 20, 1930.

Three cases of the occipital type are described in the literature. (The report of a fourth case belonging to this group is not reliable.)

Most of the reports of this congenital anomaly belong to the older literature, and quite frequently the descriptions are somewhat unsatisfactory. In general, however, a review of the literature gives us the following picture: craniopagus is a condition usually incompatible with life, although the first case is reported to have lived ten years. This is the exception, however. Most of the cases, even if born at full term, are stillbirths and at most live only a short time, several hours or a few days. The malformation may be restricted to the skull and its contents, or may be accompanied by anomalies in other parts of the body. The examination of the central nervous system has in the past been restricted to the gross anatomy. Usually the brains are described as being separate. Histological studies have been omitted, the reasons probably being that, first, most of the cases have been reported in the older literature; second, in a number of the more recently described craniopagi, the brain is said to have been in a state of decomposition and not suitable for histological examination. In the case which I wish to report the central nervous system of one of the children was quite well preserved, and detailed histological examination was possible. It is because of this, therefore, that the case is presented.

The two children forming the craniopagus belonged to triplets which were born at full term. The mother, a primipara, was physically and mentally normal, and as far as is known there was nothing remarkable in the family history. The third member of the triplet was born dead, and I had no opportunity to examine it. The craniopagus lived for five days and the postmortem was performed several hours after death. As is seen from the picture (Fig. 1) they give the impression of full term infants. The hair and nails were well developed, and there were no malformations in any other part of the body.

The right parietotemporal part of the skull of one of the infants was grown together with the right parietal region of the other, so that the two children faced opposite directions, one facing left, and somewhat upwards, the other looking to the right. We shall call the first one "A," and the second one "B."

Skull of Child "A"

The base of the skull is asymmetric. The left side is practically normal. The three fossae are well recognizable, although they are somewhat accentuated, corresponding to a similar enlargement of the brain. The frontal, parietal and occipital bones of this side are essentially normal. The right side of the skull presents a different condition. The anterior and middle fossae are confluent, forming one large depression in which the markedly deformed right hemisphere lies. The borders of the posterior fossa can not be followed. The right frontal bone is somewhat smaller than the left. All that remains of the right temporal bone is a very narrow strip at the base of the skull, and the parietal bone is entirely absent, leaving a large defect. The dura shows no malformations except for an opening in the parietal region corresponding to the defect of the skull. The cranial bones of Child "B" are normally developed with the exception of the right parietal bone in which a large defect is present. The base of the skull is quite well developed. It is through this defect that the right hemisphere of Child "A" extends into the cranial cavity of Child "B." The dural defect of "B" is similar to that of "A." The two brains, therefore, are separate units. The leptomeninges of the two brains are also separate and show no defects.

Of the two brains, that of Child "B" has been destroyed by the growth of the brain of Child "A" to such an extent that it is impossible to study it. Only a small portion of the occipital lobe of the compressed brain could be histologically examined. The brain of Child "A," however, is well preserved and presents the following features (see Fig. 2).

Cerebrum: The right hemisphere is larger and the gyri fewer than the left. The gyri are flat and hardly recognizable, and most of the sulci are absent, thus making it very difficult to differentiate between the various lobes. Even the Sylvian fissure can not be made out with certainty. It extends down into the base of the brain and can be followed there through the whole width of the cerebrum. Another deep fissure along the distorted frontal pole may represent the central sulcus. The temporal pole can not be clearly outlined. Excepting the above mentioned Sylvian fissure, no other identifications can be seen at the base. The olfactory tract and bulbs are well developed. On the median surface one can only recognize the

calloso-marginal fissure, but no definite gyri can be traced. The left hemisphere is smaller than the right, but its structures are better defined. Here one can recognize the central sulcus, the Sylvian fissure and the inferior frontal sulcus. A number of smaller convolutions can also be seen, but are not identified. On the median surface the calcarine and callosal marginal fissures can be recognized; here too, there are a number of less well identified gyri. Although the general outline of the structures is very much like those found in the fetal brain, they differ somewhat from the full term infant, and are more like those present in a seven or eight months fetus.

Cerebellum: The cerebellum is asymmetrical, the left hemisphere being larger than the right (Fig. 2). It is only partially covered by the cerebrum and is in a developmental stage of the type found in a fetus of seven or eight months. The vermis and the two hemispheres can be clearly differentiated.

A frontal section through the brain at a level midway between the tips of the frontal and occipital lobes shows the following: The cut surface of the section is white; the cortex and white matter can be differentiated with difficulty, whereas the basal ganglia are fairly well outlined and apparently of normal contour; the corpus callosum does not show anything remarkable; in the unusually narrow white matter, several small foci of softening can be seen. The left lateral ventricle is of comparatively normal size and shape. The right lateral ventricle, however, is greatly enlarged laterally although it has a small vertical diameter. Sections through the cerebellum and medulla show the fourth ventricle to be asymmetrical, the left side being larger than the right; other abnormalities of significance are lacking.

Histological Examination

Left Hemisphere: Of the two hemispheres this is the better preserved and the more highly differentiated. The cyto-architecture here differs in a great many respects from that of the new-born child. The predominant picture in the cortex is that of a six layer type (see Fig. 3); the first layer is rather poorly supplied with cell elements. The second is dense but narrow, and gradually passes into the third layer which becomes quite distinct from the second as it is definitely poorer in cells. The fourth (the inner granular layer) is quite well defined and compact, in contrast to the fifth which again

has a rather sparse cell content. The sixth layer is not quite as definite, merging into the white matter, but nevertheless well recognizable. The agranular type of cortex is not quite as distinctly represented but is nevertheless definitely recognizable. Here also six layers can be distinguished. The first has a poor cell content as contrasted with the richly cellular second layer. This latter is wide and most probably represents a fusion of the third, fourth, fifth and sixth layers. The cortex is very well demarcated from the white matter. The line of demarcation between the granular and agranular types is also quite distinct. Outside of these two predominating types, some other fields can be more or less distinctly recognized. Thus, for instance, the anterior central cortical region can be identified by the presence of the giant pyramidal cells. Then again, the calcarine area is characterized by the widening of the fourth layer. The point of highest differentiation, and a development which comes nearest to that of a new-born child is reached in the allo cortex where the uncus and the cornu ammonis are easily recognized and do not differ from that of the full term infant.

Right Hemisphere: The architecture is very much disorganized by the intense distortion. The calcarine formation is entirely absent. It is practically impossible to differentiate the various fields, all of them being represented by a more or less uniform six layer cortex. The development of the allo cortex is much inhibited and even the usually well recognizable cornu ammonis can not be distinguished. The different convolutions are mostly only rudimentary, rather large, and flat (see Fig. 4). The different cortical layers are unevenly distributed showing at various places indentations into one another. Their cell content also is irregularly distributed. The whole picture is that of a distorted compressed brain tissue.

Basal Ganglia: The pallidum has the most normally developed architecture. Its parenchyma varies little from that of a new-born child. The striatum, however, does not show as highly a differentiated development. It contains numerous cells. The large ganglion cells are well represented. The small ones, however, still bear a resemblance to neuroblasts. A similar resemblance to the neuroblast type is found in the cells of the thalamus. The pons, cerebellum and cord show practically no difference from those found in the full term infant. The sections stained with Weigert's method reveal no myelin sheaths. The neuroglia in general disclose no tendency

to proliferation. Occasionally protoplasmic elements can be seen, such cells containing some greenish pigment. It is interesting to note that no gitter cells are found anywhere, although as it was mentioned above, there were occasionally small foci of softening in the white matter. The vascular system shows a mild proliferation. The endothelial cells are somewhat swollen and the course of the vessels shows a tendency to tortuosity. On frequent occasions one finds a fine black pigment deposit in the vessel walls. Unfortunately, no frozen sections could be successfully made, as the tissues were too soft even after a prolonged fixation in formalin. The section from the occipital lobe of the brain of Child "B" was the only part that could be histologically examined, showing the same picture as the left hemisphere of Child "A."

Discussion

In our attempt to determine the probable stage of development which has been reached by the central nervous system here described, we may be guided by the observations made in the cases of developing human beings. According to His,[7] the cortex remains uniform showing no laminar structure until the fifth month of intrauterine life. Then a gradual grouping of the neuroblasts into layers occurs. First, the fifth and sixth layers appear, and these are followed by the differentiation of the second, third and fourth. This process generally extends over the period from the sixth to eighth month, at the end of which time the six layer type is definitely recognizable. The different fields show some difference as to the time when this stage is reached. As this development goes on the agranular type of structure appears. As we know, the agranular type is the product of a change in the six layer type where the inner granular layer disappears. As an example of this type we can take the anterior central region of the new-born child. The appearance of the agranular type takes place after the formation of a six layer cortex, but does not appear at the same time in all fields. It is because of this fact that we can differentiate the different stages of development by the histological picture. In our case, for instance, in the left hemisphere we found that although pyramidal cells have appeared in the central convolution, they were as yet interspersed with a fourth granular layer; in other words, we were dealing there with a stage when the six layer type was gradually changing into an agranular type. This

would lead us to place the probable height of development of the brain at the eighth month of intra-uterine life.

The developmental stage reached by the brain, as well as the development of the rest of the body would lead one to assume that the fusion of the two skulls was the result of a late malformation (this would agree with Schwalbe's [8] views on the subject). All our observations point to the fact that we are dealing here with two distinct embryos, each one of which in itself has no tendency for developmental anomalies. The whole central nervous system seems to have started in a normal process of development, and has progressed along this line until the rapid increase in size due to the formation of the convolutions which have come up against the obstacle of the limited space available. It was here that apparently a purely mechanical compression interfered with the normal rate and direction of development and brought about the type of structure that we have described. We can see that the pressure phenomena begin to influence the development of the brain about the time of the convolutional formations because the gyri that are found here are simple, smooth and extraordinarily flat. The sulci are shallow and in places pushed out of their normal position. In some places, whole regions are either destroyed or compressed to such a degree that they can not be recognized. All of this would speak for the appearance of a marked scarcity in space at the time when development was at its highest. It is quite possible too that the fusion of the skulls appears rather late, and that at an earlier stage the two were distinctly separate. This is rendered especially probable because of the fact that different parts of the skulls have fused with one another, the right parietotemporal of one with both parietal bones of the other. This again has also been noted by Schwalbe. Just why the brain of the one child has been damaged so much more than the other can not be answered.

Summary

1. The case described belongs in the group of craniopagus parietalis.

2. The malformation is confined to the skull and brain.

3. The brain of the child "A" is asymmetrical and shows pronounced malformation of the gyri and sulci which makes the identification of the latter difficult. Many sulci are absent.

4. The general outlines of the brain are like those in a seven or eight months old fetus.

5. Histologically both the granular and agranular six layer types of cortex are present.

6. In the precentral region the fourth layer is still granular in structure, which corresponds to the development of the eighth month of intrauterine life.

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DESCRIPTION OF PLATES

PLATE 98

FIG. 1. The heads of the two infants are united in such a way that the right parietotemporal region of the one on the right is grown together with the right parieto-occipital region of the one on the left.

FIG. 2. Brain of the Child "A." The right hemisphere is larger than the left. The convolutions show pronounced malformations. The right occipital lobe is absent. The left hemisphere is better developed. The cerebellum is asymmetric, the right lobe being much smaller than the left; the cerebellum is not covered by the cerebrum. The spinal cord is normal.

1

2

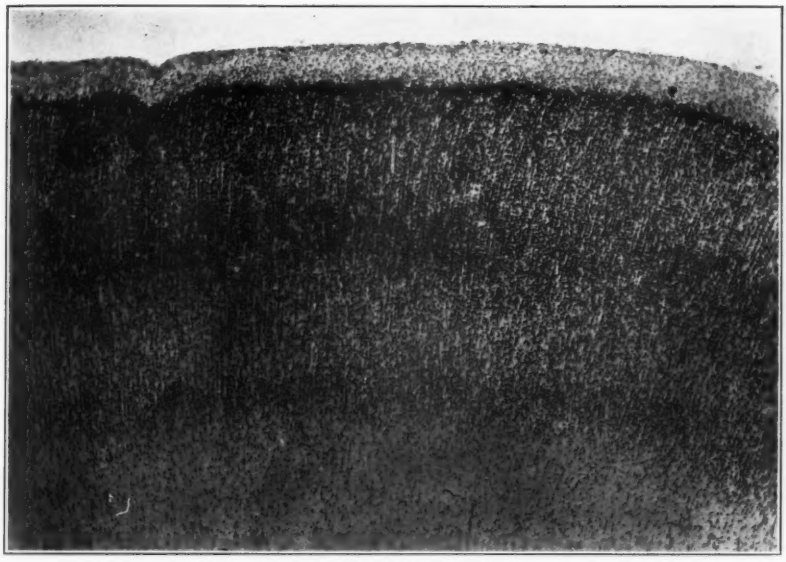
Löwenberg

Histological Study of Brain of Craniopagus

PLATE 99

FIG. 3. Shows clearly the six layer type of the fetal cortex.

FIG. 4. Six layer type clearly visible. Sulci shallow and primitive.



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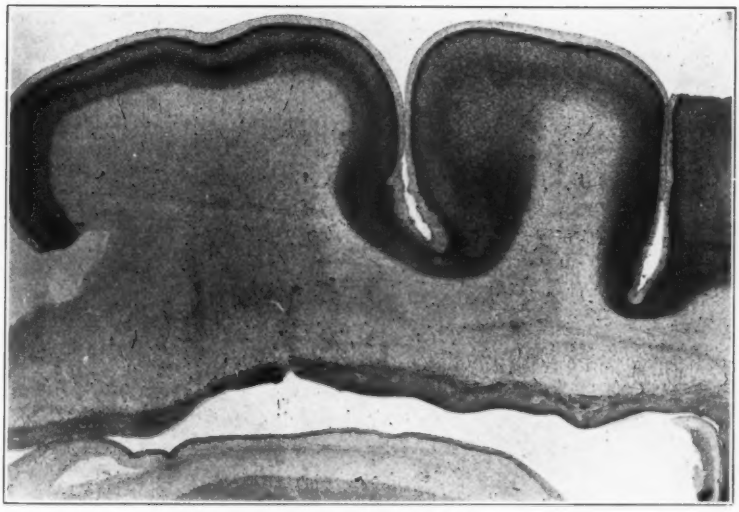

4

CONGENITAL ANEURYSM OF THE INTERVENTRICULAR SEPTUM *

Report of Two Cases

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Congenital anomalies of the heart are of not infrequent occurrence. Congenital aneurysms of the interventricular septum, however, are quite unusual, and when encountered are valued for anatomopathological studies rather than for any practical importance from a clinical standpoint. They are usually discovered incidentally at autopsy. If they give rise to symptoms during life, these are of such complicated nature that the diagnosis is rarely made correctly. The following two cases are of interest in that they were seen within one month of each other at autopsy, and neither gave any clinical evidence of its presence.

Case 1

Clinical History: G. H., a young man 24 years of age was admitted to the medical service of the Toronto General Hospital on March 12, 1928, complaining of pain in the left chest, with chills and fever for three days. Nothing further of importance for this report was elicited in the history. The physical findings were those of a severe pneumonia of the left lower lobe along with some cardiac enlargement. The patient became progressively worse until his death four days later.

Postmortem Examination

The postmortem examination revealed a confluent bronchopneumonia in the lower lobe of the left lung with partial collapse of both right and left upper lobes. A marked fibrinous pleurisy was present on the surface of both lungs, and a moderate effusion was found in the pleural cavities. A Meckel's diverticulum was seen about two feet proximal to the ileocecal valve; no other abnormalities were found in the abdominal cavity. The left kidney and the spleen were the site of numerous, small infarcts, while healed miliary tubercles were found in the spleen and liver. These organs presented the usual picture of cloudy swelling associated with severe toxemias,

* Received for publication April 11, 1930.

and in addition a marked degree of focal necrosis was present in the liver.

The heart weighed 290 gm. and measured 12.4 by 13.5 by 5.2 cm. The apex was quite sharp and was formed chiefly by the tip of the left ventricle. The epicardial fat was moderate in amount. The coronary arteries were not palpably thickened. The right heart contained a considerable amount of postmortem clot. The valvular orifices measured: tricuspid 13.5, pulmonary 6.8, aortic 6.5, mitral 10.7 cm. The valve curtains throughout were thin, translucent and pliable. Two small fatty plaques were present in the free cusp of the mitral valve. The undefended space in the septum was anomalous in its appearance, there being a marked aneurysmal pouching extending from the left ventricle to the right auricle. It arose 9 mm. below the common point of attachment of the right and left posterior aortic valve cusps to the aortic wall. It was roughly circular in contour, and measured 2 by 2 cm. in width and 1.8 cm. in depth. Several fibrous trabeculae extended from the periphery of the pouch toward its central portions. These were of approximately the same thickness as the ring of fibrous material about the periphery, but they were considerably thicker than the central areas of the pouch, which were glistening, thin-walled and translucent. The aortic valve cusps were quite competent. The sinuses of Valsalva presented no abnormalities. On looking through the aortic ring into the heart, the aorta was seen to be directed toward the right ventricle. The muscular septum protruded into the aortic vestibule and the membranous septum was almost horizontal instead of vertical. The endocardium of the left ventricle was smooth and glistening. The aneurysmal pouching extended in a rounded manner through the medial cusp of the tricuspid valve at its junction with the anterior cusp. The latter of these formed the left lateral boundary of the pouch, which measured 2.3 by 1.8 cm. Its surface was smooth and glistening. The tricuspid valve cusps were thin and pliable and no thickenings of their free margins were seen. The foramen ovale was patent in a slit-like manner along the anterior margin of the fossa ovalis. The wall of the left ventricle measured 1.7 cm. in thickness, and that of the right ventricle measured 5 mm. The cut surface of the heart muscle was deep pinkish red in color. The surface was glistening in appearance and no whitish streaks were seen. The coronary arteries arose in normal position. They were thin-walled throughout, and presented no areas of atheroma.

Case 2

Clinical History: J. R., a man, aged 60 years was admitted to the surgical service of the Toronto General Hospital on March 1, 1928, complaining of difficult micturition and almost complete retention of urine. History and physical examination beyond the genito-urinary tract were negative. One month later, following the resection of a vesical diverticulum, the patient developed peritonitis and died.

Postmortem Examination

At autopsy a foul-smelling peritoneal exudate was found which had its source in the urinary bladder. In addition to congenital cardiac anomalies, the examination revealed a congenital absence of the gall bladder and cystic duct, together with abnormal lobation of the right lung, the presence of accessory spleens and supernumerary renal arteries. No noteworthy pathological changes were found elsewhere.

The heart weighed 365 gm. and measured 15.2 by 12.5 by 5.2 cm. The right heart was somewhat dilated, but the left ventricle was contracted. The heart presented two apices and the right border was sharply concave. The main apex was sharp and was formed by the tip of the left ventricle. The apex of the right ventricle stood out at right angles from the septum and formed a firm mass near the line of the interventricular groove. The epicardial fat was moderate in amount. There was a small, white, localized thickening of the pericardium of the left ventricle. The base of the right ventricle felt quite thick and projected outward from the septum for a distance of 12 cm. on the posterior surface, and 9.8 cm. on the anterior surface. The pulmonary arteries were very large, measuring 9.2 cm. in circumference. The superior vena cava was quite small, barely admitting an index finger. The cavities of the heart contained small quantities of postmortem clot. The valvular orifices measured: tricuspid 12.5, pulmonary 7.3, aortic 8.2, mitral 10.2 cm. The tricuspid valve curtains presented a slight amount of diffuse thickening of their free margins. The medial cusp of this valve was quite thin. A sacculated aneurysm occupying the region of the undefended space of the interventricular septum projected into the right ventricle for a distance of 2 cm. at the common point of attachment of the medial and anterior cusps. The valve curtain over the aneurysm was slightly thickened and a small projection was present, which simulated a fourth valve cusp. No chordae tendineae were

attached to this rudimentary cusp. The aneurysmal sac was multilocular in appearance. Its surface was irregularly thickened by fibrous trabeculae running inward from its periphery. As in the previous case, there were translucent areas in its central portion. It measured 2.5 by 1.8 cm. as seen from the right ventricle. The tricuspid valve was apparently competent. There was a small fibrous band running across the cavity of the right auricle. The foramen ovale was closed. The pulmonary valve consisted of two cusps only. These were large, and one of them presented a fibrous band joining its free margin to the wall of the pulmonary artery at a point 1 cm. from the insertion of the base of the cusp into the wall of this vessel. This formed a rudimentary third cusp which measured 1 cm. in width. A small ridge was found in the base of the sinus immediately below this fibrous band. The width of the larger curtain was 4.5 cm., and the smaller curtain was 2.4 cm. The free margins of these curtains were slightly thickened and somewhat roughened. The aortic valve cusps were much larger than usual and presented numerous fenestrations. The right curtain measured 3.5 cm., the left 2.5 cm., and the posterior 2.9 cm. in width. The right and posterior cusps were fused in an irregular manner, but no thickening was present at their point of fusion. In looking through the aortic ring into the heart, the aorta was seen to be directed toward the right ventricle. The muscular septum projected into the aortic vestibule and the membranous septum was horizontal in direction instead of vertical, its floor being formed in part by the floor of the aneurysm. The mouth of the aneurysmal sac opened at a point 2.5 cm. below the common point of attachment of the posterior and right valve cusps. It measured 2.8 by 1.6 cm. in width, 3.8 cm. in depth and was somewhat oval in shape. It presented a multiloculated appearance, with small pouches extending outward from the largest one, which occupied the greater portion of the undefended space of the interventricular septum and projected into the right ventricle beneath the medial cusp of the tricuspid valve. Its surface was a glistening white color, and there was no evidence of a previous inflammatory process. The sinuses of Valsalva were very large, but normal in general contour, except that the base of the right sinus was formed by myocardial tissue. This sinus extended below the point of origin of the wall of the aorta for a distance of 5 mm. The mitral valve curtains showed a slight degree of diffuse thickening of their free margins, and the septal cusp presented a pouch measuring

1.5 by 1.3 cm. in diameter, which projected for a distance of 1.2 cm. into the left auricle. The coronary arteries arose slightly above the level of the aortic ring. Their mouths were large and funnel-shaped. They did not lie over the centers of the sinuses from which they arose, but were situated at the adjacent margins of the respective sinuses. These vessels were thin-walled throughout, presenting only one or two small, yellowish plaques beneath their intima. The wall of the left ventricle measured 1.8 cm. in thickness, and that of the right ventricle measured from 3 to 8 mm. The cut surface of the heart muscle was deep pinkish-red in color, showing numerous red stipplings. A localized area of fibrosis, measuring 2.2 by 1.5 cm. in diameter, was found on the lateral surface of the left ventricle near the tip. The wall of the ventricle at this point presented a small area of bulging, measuring 5 mm. in diameter. The large size of the aneurysm in this case, in the absence of any evidence of valvular incompetence, was remarkable.

Discussion

Other cases of aneurysm of the interventricular septum have been reported by Mall,[1] McCallum,[2] Merkel,[3] Tate,[4] Guccione[5] and Goehring.[6]

Those of Mall and Merkel were similar in appearance and localization to our first case. In that of the former a cystiform aneurysm was present at the junction of the aorta with the left ventricle. It involved the membranous septum, burrowed into the anterior part of the medial cusp of the tricuspid valve, and projected into the right antrium. Merkel's case presented an aneurysm from the left ventricle into the medial cusp of the tricuspid valve, as well as a partially patent interventricular septum. On the other hand, those of McCallum and Tate more nearly simulated the findings of our second case. In that of the former the pars membranacea septi bulged into the right ventricle, forming a sacculated projection beneath the tricuspid valve. The mouth of this sac was seen just below the aortic valves. In that of the latter a trumpet-shaped tube of membrane was found projecting into the right ventricle, at the junction of the attached margin of the septal and infundibular segments of the tricuspid valve. On passing a probe along this hollow membranous tube, it was seen to communicate by a rounded opening with the lower part of the right cusp of the aortic valve. It appeared to have commenced as a left-sided saccular projection into

the right ventricle, through the undefended space which later became perforated.

The case reported by Guccione was somewhat akin to Case 1, but possessed certain features which necessarily place it in a separate class. A distinct funnel-shaped protuberance of the wall emerged immediately above the septal cusp of the tricuspid valve. The sharpened apex of the aneurysm was directed downward toward the ventricle and was partially covered by recent thrombi. On removing these, the remaining portion of the aneurysm presented a transverse opening, which could be penetrated by a probe. The protuberance was covered by thickened endocardium and was pasty in consistency. The aortic valves were markedly distorted by recent vegetations and old scars. Between the thrombi on the posterior cusp and the base of implantation of the anterior cusp of the mitral valve, a fissure was observed in the interventricular septum. On removing the clots filling this rupture, a funnel-shaped diverticulum was seen, which was also filled with thrombi. This corresponded with the swelling in the right antrium, immediately above the septal cusp of the tricuspid valve.

The etiology of these aneurysms is a matter of some disagreement. Guccione believes that the primary production of the aneurysm is due to endocarditis. This, he states, is usually an extension of the process from the mitral or aortic valve cusps, which is facilitated by their proximity to the membranous septum. Following erosion of the endocardium over the pars membranacea, the blood tends to infiltrate into the lower stratum of the septum, because the pressure is higher in the left ventricle than in the other cavities of the heart. Thus, Guccione concludes, they must be considered as "true dissecting aneurysms, not only because they are as a rule acute, but also on account of their evolution." Histological examination in his case further adds to the strength of his views. He considered that the aneurysm was due to recurrent chronic aortic endocarditis, with a terminal acute attack which resulted in ulceration of the sac and the subsequent death of the patient.

Mall and Goehring, on the other hand, consider that these aneurysms are the result of congenital malformations. The latter points out that it is difficult to understand how an aneurysm can penetrate the dense fibrous ring of the atrioventricular orifice, and burrow into the delicate fibrous tissue of the cusp of a fully developed heart. He suggests that they occur some time after the completion of the

septum, but before the replacement of the musculature of the cusp by fibrous tissue.

Mall suggests that these aneurysms result from a failure of the inferior or fleshy portion of the interventricular septum to shift sufficiently far to the right. The aorta during the course of development of the heart, shifts from the right side of the heart to the left. According to Mall, after gaining its permanent position, the inferior septum blends with its right side, forms the membranous septum, and completes the wall between the left and right ventricles. This is in accordance with the views of Tandler [7] and Jordan,[8] who state that the membranous portion of the septum results from the fusion of the dorsal endocardial cushion dividing the atrioventricular canal, the proximal end of the aortic septum and the cephalic margin of the muscular interventricular septum. Mall points out that in the cases described by McCallum, Zahn, and Rokitansky, the muscular septum protruded into the vestibule of the aorta. This is also true of our cases. The aorta in the second case would seem to be communicating with the right ventricle rather than the left ventricle; this is also seen in the first case, but to a lesser degree. We may, therefore, conclude that in our cases, as in those of Mall and others, the aneurysm resulted from an embryonic arrest of development in which the inferior or muscular septum did not move sufficiently far to the right, but remained in the vestibule of the aorta. As a result of this misplacement the membranous septum developed in a horizontal plane instead of in its normal perpendicular one. This alone weakens it in every way, and with or without a possible defective texture could result in the production of an aneurysm. Normally the membranous septum lies below the tricuspid valve, but a slight distortion may displace the origin of the medial cusp on the membranous septum and result in the invasion of the valve, such as occurred in Case 1, whereas in Case 2 the aneurysm projected beneath the medial cusp of the tricuspid valve in the position where it would be expected.

The absence of any evidence of endocarditis in our cases, for one would hesitate to pronounce the thickenings of the mitral and tricuspid valve cusps in the second case as sequelae of a previous endocarditis, together with the failure to elicit any history of cardiac involvement, further strengthens the view that they are the result of congenital malformations, rather than the terminal results of endocarditis. Indeed, it seems possible that the acute case of

Guccione may have resulted from the superimposing of an endocarditis upon a congenital anomaly. Such anomalies are notorious asd loci for infection.

The presence of associated anomalies in the heart and other organs in the second case is of great interest, and adds additional weight to the theory of the congenital nature of these aneurysms.

Summary

1. The clinical and pathological findings in two cases with congenital aneurysm of the interventricular septum are here reported.

2. The absence of clinical signs and symptoms in both cases, wherein marked distortion of the normal anatomy was present, is remarkable.

3. A critical study of our cases adds further evidence in favor of these anomalies being congenital malformations, rather than the terminal results of endocarditis.

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DESCRIPTION OF PLATES

Plate 100

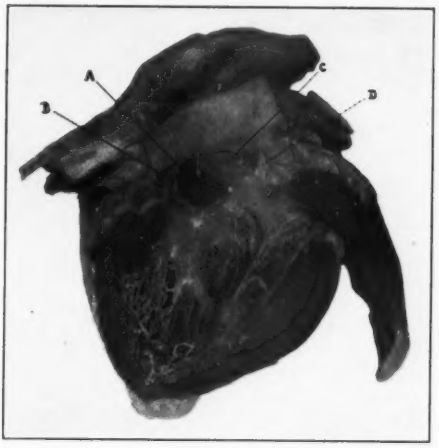
Fig. 1. Left side of heart (Case 1). (A) Aneurysm. (B) Anterior aortic valve cusp. (C) Right posterior aortic valve cusp. (D) Left posterior aortic valve cusp.

Fig. 2. Right side of heart (Case 1). (A) Aneurysm bulging through medial cusp of tricuspid valve.

Fig. 3. Left side of heart (Case 2). (A) Coronary orifices. (B) Aneurysm. (C) Anterior cusp of mitral valve.

Fig. 4. Right side of heart (Case 2). (A) Medial cusp of tricuspid valve. (B) Aneurysm. (C) Rudimentary fourth valve cusp. (D) Anterior cusp of tricuspid valve. (E) Inferior cusp of tricuspid valve.

Fig. 5. Right side of heart viewed from below tricuspid valve (Case 2). (A) Aneurysm. (B) Pulmonary artery.



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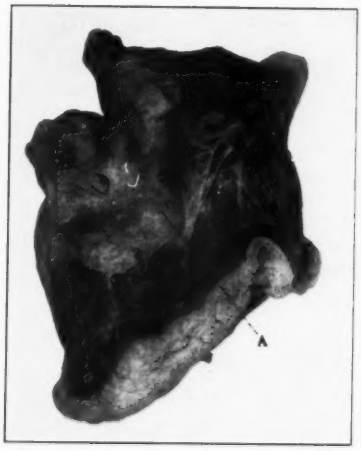

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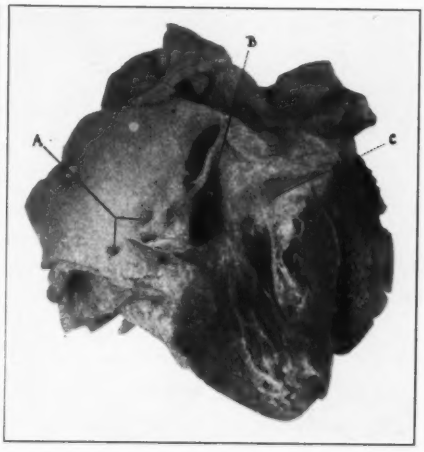

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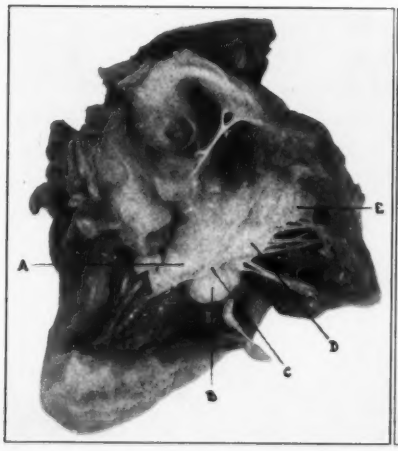

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5

RENAL LESIONS WITH RETENTION OF NITROGENOUS PRODUCTS PRODUCED BY MASSIVE DOSES OF IRRADIATED ERGOSTEROL *

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INTRODUCTION

Since the work of Hess,[1] Steenbock,[2] Hess and Windaus,[3] and others, showing that by the administration of irradiated ergosterol in extremely small doses it is possible to prevent or cure rickets, there has been much interest in the effect of this substance upon all conditions concerned with the metabolism of calcium. In addition, a number of investigators have studied the effects of very large doses of irradiated ergosterol upon experimental animals. The work of Pfannenstiel,[4] Kreitmair and Moll,[5] Klein,[6] Rabl,[7] and of Smith and Elvove [8] is especially noteworthy. They have shown that the administration of massive doses of irradiated ergosterol to rabbits and other susceptible animals leads to rapid loss of weight, cachexia and death. There is also a marked increase in blood calcium, and at autopsy extensive calcium deposits are found in the arterial walls, especially the aorta, in the heart muscle, stomach wall, lungs and kidneys.

The kidney lesions reported by previous investigators were chiefly in the parenchyma rather than in the blood vessels. Kreitmair and Hintzelmann [9] found, in rabbits, calcification in the membrana propria, around the convoluted tubules, also in the glomeruli, and sometimes in the vessels leading to them, with calcium deposits within the lumina of some of the straight tubules. Rabl described calcification of the basal membranes and epithelium of the tubules of the cortex in mice, with calcification of the vessel walls "in places." Smith and Elvove likewise found calcification and calcium casts in the tubules and in addition spoke of an "interstitial and glomerular nephritis." No calcium deposits were seen by them in the kidney vessels.

There is general agreement that the blood calcium rises markedly as a result of large doses of irradiated ergosterol. Klein noted also a

* Received for publication April 1, 1930.

low serum protein and a high albumin-globulin ratio. The non-protein nitrogen averaged 57 mg. per 100 cc. of blood.

The present paper deals with a detailed histological study of the lesions produced in the kidneys of rabbits by the administration of massive doses of irradiated ergosterol. The effect of these lesions upon kidney function, as demonstrated by the retention of nitrogenous products in the blood, was also studied.

Material and Methods

Sixteen young rabbits, averaging about 2300 gm. in weight, were used. A preparation of "Irradiated Ergosterol, 1000 D" * having one thousand times the antirachitic potency of cod liver oil was administered by stomach tube, in doses of from 3 cc. to 10 cc. at intervals of from one to four days, except for one experiment (No. 40, Table I), in which the average interval was five days. Three pairs of animals were killed on the fourth, sixth and eighth days respectively, in order to determine the early changes in the kidneys. The remaining ten were allowed to die from the effects of the irradiated ergosterol. A series of eight control animals received the non-active solvent oil used in the preparation of irradiated ergosterol employed, in similar doses.

From the control animals and the ten animals which received fatal doses of irradiated ergosterol, blood samples were taken at intervals and the non-protein nitrogen, urea nitrogen, creatinine and uric acid determined by the methods of Folin *et al.*[10] Hemoglobin was estimated by the Tallqvist method. The urine was tested for albumin, on the days when blood samples were taken, by the use of nitric acid.

Sections from the organs were fixed in an alcohol-formalin mixture (9 parts of 95 per cent alcohol to 1 part of 40 per cent formalin), in 10 per cent formalin, 95 per cent alcohol, Helley's fluid and in Zenker's fluid. A portion of the tissues fixed in formalin was embedded in celloidin and stained either with hematoxylin and eosin or by the silver method of von Kossa. Another portion of the formalin-fixed tissues was embedded in paraffin and stained with hematoxylin and eosin. In order to demonstrate fat, frozen sections of formalin-fixed material were stained with Sudan IV. Other sections

* This preparation was supplied through the courtesy of Mead, Johnson & Co.

of the same material were stained with methyl violet as a test for amyloid. The tissues fixed in Zenker's fluid were embedded in paraffin and stained with eosin-methylene blue. Weigert's elastic tissue stain was used on both formalin and Zenker-fixed materials. The calcium deposits were identified by their solubility in acid and by the following histological criteria, using principally formalin-fixed material: (1) When stained with hematoxylin and eosin the precipitated calcium appeared as a dark blue, coarsely granular material. (2) After treatment with a silver nitrate solution and counterstaining with a 0.5 per cent solution of basic fuchsin the deposits assumed a deep brownish black color.

Results

Toxic Manifestations: A demonstrable loss of the rabbit's body weight occurred within a few days after the administration of large doses of irradiated ergosterol was begun (Table I). Cachexia, loss of appetite, and oftentimes diarrhea developed as the irradiated ergosterol was continued. The hemoglobin showed a slight tendency to fall. The urine invariably contained a large quantity of albumin during the last day or two of life, and at the same time there was usually some retention of urine.

Of the control animals (Table II), four maintained a constant weight and two showed only a very gradual decline. The other two (Nos. 17 and 21), showed a considerable loss of weight and developed diarrhea. The urine showed small amounts of albumin on three occasions, a finding which is not unusual in apparently healthy rabbits.

Pathological Description of the Kidneys

This report will be confined to the study of the kidneys of the twenty-four test animals, and the general pathological studies will be published subsequently.

Gross: The kidneys from the animals dying of irradiated ergosterol poisoning were found occasionally to be slightly reduced in size, but they always appeared normal in color, shape and consistence. After sectioning, however, definite macroscopic changes were seen in the cortex and medulla of the kidneys from the animals surviving the longest. These changes were not apparent in the animals

living for a shorter period despite the fact that comparable doses of irradiated ergosterol were administered. This suggests that the time element is a very important factor in the production of the lesions. Regardless of whether or not gross changes were present, the capsule of each kidney was thin, stripped with ease and left a smooth glistening surface. Innumerable small, but easily visible, grayish brown areas (calcium) were studded throughout the cortex of the advanced cases. In the same cases a grayish brown concentric ring (calcium) approximately 0.1 cm. in thickness lay in the medulla, parallel to the curve of the cortex and 0.2 cm. medial to its inner margin. The cortex in all cases was of essentially normal thickness, and was well demarcated from the medulla.

Some sclerosis was observed in the renal arteries of the animals showing gross deposits of calcium in the cortex and medulla. On the other hand, the animals that died the most rapidly from ergosterol poisoning, but without gross changes in the cortex or medulla, showed little or no changes in the renal arteries. Rarely, slight thickening of the walls of the arteries was detectable when it was difficult to determine grossly if calcium deposition had occurred in the kidney.

The kidneys from the control animals, and from the animals receiving irradiated ergosterol for less than nine days, appeared normal in every respect.

Microscopic Description of the Kidneys

1. *Arteries:* The glomerular arterioles and the interlobular arteries (middle-sized and small arteries) in all the rabbits dying from irradiated ergosterol were sclerosed. The larger branches of the renal arteries usually appeared normal. The changes were focal in distribution and were almost entirely limited to the cortex and to the adjacent portion of the medulla. Hyalinization and calcification were the most characteristic changes in the walls of the affected vessels. The histological picture of the larger interlobular arteries was somewhat different from that of the glomerular arterioles. In the interlobular arteries the media was chiefly involved, whereas in the glomerular arterioles the media was principally involved, but often the other layers were hyalinized and fused. In the most damaged interlobular arteries the media and intima were fused in a manner comparable to the arterioles. The adventitia of the interlobular

arteries was rarely involved. In both the arterioles and the interlobular arteries showing advanced changes, the walls were thickened with resulting diminution in the caliber of the lumina. Frequently the contour of the vessels was altered and the lumina were eccentrically placed. Many of the lumina were reduced to mere slits, or even entirely obliterated. In such cases the sites of former lumina were occupied by fused masses of necrotic material, calcium salts and cellular débris. In some instances polymorphonuclear and endothelial leucocytes were found in this material.

Calcium and Hyalin: Large and small irregularly shaped masses of calcium were deposited in the walls of the interlobular arteries. In the arterioles the deposits were smaller and less numerous. The calcium deposits in the arteries were located chiefly in the media and internal elastic lamina. In some vessels these deposits in the media were sharply separated from one another by normal tissue, while in others they fused to form a calcified ring encircling the lumina. These deposits occurred both in normal appearing and necrotic areas of the vessel walls. After decalcification many of the areas formerly occupied by calcium appeared either as hyalinized material or as degenerated regions containing vacuolated cells without nuclei or with pyknotic nuclei.

In many instances the calcification and hyaline formation were so intimately related that it was impossible to differentiate sharply between them. At times, however, a definite layer of hyaline material lay internal to the calcium deposits. After removal of the calcium the bluish pink-staining hyaline material became more prominent. It was deposited in areas of irregular size and shape in the media and subintimal portion of the interlobular arteries, and as a subendothelial band of irregular thickness and distribution in the glomerular arterioles. This irregular distribution was especially well shown in longitudinal sections of the affected vessels. The tissues immediately adjacent to the hyaline deposits were well demarcated from them and appeared normal. Instances were observed in which the afferent arteriole was normal, but the interlobular artery leading to it was undergoing hyalinization. Areas of necrosis were occasionally found in the hyalinized regions. In general the amount of hyalinization paralleled the degree of vessel involvement.

Fat: Many of the severely damaged vessels were heavily laden with droplets of fat. These deposits were larger and more numerous

in the interlobular arteries than in the glomerular arterioles. However, strikingly different amounts of fat occurred in vessels of similar caliber and with a comparable degree of sclerosis. In the interlobular arteries the droplets were located chiefly in the media, while in the glomerular arterioles, the subintimal regions were especially rich in fat. These fat deposits were for the most part found in arteries showing marked calcification. However, some of the calcified vessels were free from fat, while occasionally there were scattered droplets of fat even in the walls of otherwise normal appearing vessels. Large amounts of fat filled the sites of obliterated lumina and the areas of necrosis within some of the vessel walls. The amount of fatty material was roughly proportional to the degree of general vessel involvement.

Elastic Tissue: There was marked thickening of the internal elastic lamina of many of the interlobular arteries. Thickening of the layers and splitting of the fibers was common. In general the external elastic lamina was normal. Occasionally, however, some of the individual fibers were irregularly thickened. The elastic tissue of the glomerular arterioles, when these were markedly involved, was indistinguishably fused with the adjacent portions of the wall.

2. *Veins:* The middle-sized and smaller veins showed changes similar to those in the corresponding arteries, but these changes were neither so numerous nor so marked as in the arteries.

Both the arteries and veins of the control animals were normal in every respect, as were those from the animals killed within eight days after first receiving irradiated ergosterol.

3. *Tubules:* Extensive pathological changes occurred in the renal tubules of all animals that received irradiated ergosterol. Structural changes were much more apparent in the convoluted tubules and the loops of Henle than in the collecting tubules. The most conspicuously involved tubules were in the vicinity of the clusters of affected vessels described above. In the severely damaged kidneys there were focal areas in which the tubules showed marked calcification and hyalinization and thickening of the basement membrane. In a few of the most severely damaged kidneys, the majority of the tubules were markedly atrophic. In the less damaged kidneys the majority of the tubules were undergoing cystic dilatation, with little or no thickening of the basement membrane. There were many areas containing tubules in various stages of transition

between simple cystic dilatation and the more advanced condition of calcification and thickening of the basement membrane. Calcium was deposited in and near the basement membrane of many of the severely damaged tubules, and to some extent within the degenerated epithelium. The calcium appeared as large or small isolated masses of irregular contour which often fused to encircle the lumina. The transition was abrupt between areas of calcification and immediately adjacent normal tissue. Frequently large, laminated, often oval-shaped masses of calcified and hyalinized material occurred within the thickened basement membrane. These masses indented the tubular walls and many times invaginated the walls into the lumina. A thickened, coarse, irregular zone of almost transparent bluish pink, hyalinized material often lay between the epithelium and the basement membrane. At times this layer was as thick as the original diameter of the tubular epithelium. Where the calcium deposits and hyaline changes were most marked the tubules became narrowed, distorted, and at times difficult to identify. In the severely damaged tubules the epithelium usually was degenerated, but in some of them it remained practically normal. The early changes in the epithelium consisted in hyaline droplet degeneration with scattered droplets of fat, or else swelling of the cells resulting in pronounced narrowing or even occlusion of the lumina. In the animals living the longest, however, the cells were markedly flattened and contained relatively few fat droplets. The tubules were often distended with large hyaline casts containing calcium.

The collecting tubules showed to a much less degree the changes described in the convoluted tubules and in Henle's loops. However, the most noticeable abnormality was the presence of large hyaline casts containing calcium. They distended the tubules and compressed the lining epithelium.

The tubules in the kidneys of the control animals were normal, as were those of the animals killed soon after the administration of irradiated ergosterol was begun.

4. *Glomeruli:* In the severely damaged kidneys the glomerular capsules were often thickened, hyalinized and calcified. In the kidneys injured but moderately or slightly, the capsules almost always appeared normal. The pronounced lesions in the glomerular capsules were located near groups of severely damaged vessels and tubules. The calcium occurred in irregular deposits in and near the

basement membrane. They either took the form of isolated deposits of variable size separated by normal appearing tissue, or fused to form crescentic masses. After removal of calcium there remained either areas of hyalinization or regions of vacuolization containing pyknotic nuclei and cellular débris. An irregularly thickened bluish pink layer of hyaline material lay between the basement membrane and the epithelium. This hyaline band sometimes extended around the capsule and became continuous with the hyaline layer of the tubule. At times the glomerular capsule was somewhat shrunken. In the capsules damaged to the greatest extent there were considerable deposits of fat, while scanty deposits were found in the capsules only moderately injured. A few of the tufts contained focal deposits of calcium but the great majority were normal. There did not seem to be a close relationship between the amount of blood in the glomerular tufts and the degree of vessel damage.

The glomeruli in the kidneys of the control animals and those killed early were normal.

The interstitial tissue was essentially negative in the kidneys of all animals.

Chemical Constituents of the Blood

The effect of the administration of irradiated ergosterol upon the nitrogenous constituents of the blood can best be appreciated by considering first the concentration of these substances in the blood of the control rabbits. As shown in Table II, the non-protein nitrogen averged 39 mg. per 100 cc., only two blood samples showing more than 43 mg. per 100 cc. The urea nitrogen averaged 15 mg. per 100 cc., creatinine 1.6 mg., and uric acid 1 mg. The value for uric acid was about one quarter as great as is normal for human blood.

The bloods of the animals receiving fatal doses of irradiated ergosterol all showed a rise in the nitrogenous constituents during the last day or two of life, the terminal values for non-protein nitrogen ranging from 67 mg. to 222 mg. per 100 cc., as shown in Table I. The urea showed a comparable but proportionately greater rise, so that at death the urea nitrogen amounted to about 65 per cent of the non-protein nitrogen, whereas in the normal animals it was only about 35 per cent. This same phenomenon occurs commonly in human beings, as a result of nephritis.[11] The blood creatinine in-

creased to a rather less degree than the non-protein nitrogen and urea, while the uric acid never rose to more than twice its original level and in several instances showed scarcely any rise at all. This last finding is in accord with Folin's observation [12] that herbivorous animals excrete uric acid with extraordinary efficiency.

The kidneys of some of the rabbits showed much more marked calcification than those of others, and in turn it is evident from the data given in Table I that the height of the non-protein nitrogen at death corresponded quite closely with the degree of calcification and general kidney damage. It is probable that the animals which showed relatively less kidney involvement *post mortem* died on account of lesions in other organs, while in those showing the most marked involvement of the kidneys, uremia was at least an important contributory cause of death. It is noteworthy that in all cases the retention of nitrogenous products occurred only in the last few days of life, after the kidney damage had become severe.

In order to determine the effect of extreme cachexia upon the chemical constituents of the blood discussed above, two rabbits in the last stages of tuberculosis were studied. The nitrogenous constituents were not found to be elevated.

Discussion

It has been shown in the experiments reported here that excessive doses of activated ergosterol administered over a period of from nine to sixty days produced marked histological changes in the renal vessels, tubules and glomerular capsules. The media of the interlobular arteries and the entire walls of the glomerular arterioles were often sclerosed, and both types of vessels contained prominent subendothelial deposits of hyaline material. In addition the internal elastic lamina of the interlobular arteries was thickened, and interweaving and splitting of the fibers occurred. The basement membrane of the tubules was markedly thickened and large areas of hyalin lay between it and the tubular epithelium. In some animals marked tubular atrophy occurred. The basement membrane of the glomerular capsules was likewise irregularly thickened, with large deposits of hyaline material between it and the capsular epithelium. Calcification was marked in isolated areas in and near the basement membrane of the tubules and of the glomerular capsules. The same

TABLE I

Effect of Massive Doses of Irradiated Ergosterol ("Super-Acterol") upon Rabbits

Exp. No.	Duration	Total dose	Number of doses	Day of experiment	Weight of animal	Albumin in urine	Hemoglobin	Blood				Degree of calcification of kidney vessels
								Non-protein nitrogen	Urea nitrogen	Creatinine	Uric acid	
	days	*cc.*			*gm.*		*per cent*	*mg. per 100 cc.*	*mg. per 100 cc.*	*mg. per 100 cc.*	*mg. per 100 cc.*	
40	60	106	12	1st	2850	0	70	67	38	2.8	1.6	
				43d	2680	0	70	48	27	1.8	0.9	
				59th	2400	..	65	120	100	2.5	2.0	
				60th	2250	..	65	222	163	2.8	2.0	++++
37	13	40	4	1st	1830	0	65	44	17	2.1	0.7	
				13th	1320	++++	..	169	115	4.5	1.3	++++
16	14	97	14	1st	2080	0	60	36	16	1.7	0.9	
				3d	1980	0	60	29	14	1.2	1.3	
				7th	1920	0	60	38	13	1.8	1.0	
				10th	1790	0	60	38	17	1.7	0.7	
				14th	1680	++++	60	160	111	5.2	1.7	++++
33	19	71	8	1st	2765	0	60	53	20	3.3	1.1	
				14th	2200	++++	70	61	32	3.0	1.3	
				16th	1980	++++	..	65	38	4.2	1.6	
				19th	..	..	..	156	115	4.0	1.5	++++
34	14	69	9	1st	2890	0	70	52	26	1.6	3.2	
				14th	2340	..	..	154	95	4.5	4.9	++++
39	11	30	3	1st	2480	0	70	60	25	3.2	0.8	
				11th	2020	++++	65	115	76	5.5	1.4	++++
31	15	66	15	1st	2715	0	70	36	12	1.7	0.6	
				11th	2105	..	70	52	28	2.9	0.5	
				15th	1980	+++	70	80	43	2.2	0.7	+++
29	12	57	12	1st	1920	0	70	42	17	1.7	0.5	
				11th	1675	++++	65	76	46	3.0	1.0	+++
20	9	85	9	1st	1830	0	60	48	15	1.8	0.5	
				3d	1760	0	60	31	15	1.2	0.8	
				6th	1620	0	55	40	15	1.8	0.4	
				9th	1460	0	50	75	42	2.9	0.8	+
30	12	55	12	1st	1920	0	55	37	20	1.7	0.5	
				12th	1440	++++	..	67	32	2.8	0.8	++
Average at death								127	84	3.7	1.6	

TABLE II

Effect of Inert Solvent Oil upon Rabbits

Exp. No.	Duration	Total dose	Day of experiment	Weight of animal	Albumin in urine	Hemoglobin	Blood				Description of kidneys
							Non-protein nitrogen	Urea nitrogen	Creatinine	Uric acid	
	days	*cc.*		*gm.*		*per cent*	*mg. per 100 cc.*	*mg. per 100 cc.*	*mg per 100 cc.*	*mg. per 100 cc.*	
28	44	325	1st	2360	..	..	..	..	..	..	
			14th	2170	o	70	30	14	1.7	0.8	
			28th	1950	o	65	39	18	3.0	1.0	
			44th	1820	o	65	36	15	2.1	0.5	Normal
19	33	250	1st	2810	o	60	38	11	1.8	2.0	
			4th	2770	o	60	35	9	1.7	1.6	
			7th	2610	o	60	43	17	1.7	1.6	
			31st	2440	o	60	40	18	2.3	1.6	Normal
42	21	210	1st	2050	o	70	43	9	1.5	0.8	
			21st	2120	+	70	34	9	1.5	1.0	Normal
43	21	210	1st	2350	o	70	32	13	0.9	1.0	
			21st	2400	o	70	32	11	1.5	0.6	Normal
44	21	210	1st	2450	o	65	37	18	1.1	0.8	
			21st	2500	++	65	32	11	1.5	0.8	Normal
45	21	210	1st	2150	o	60	39	20	1.3	1.1	
			21st	2180	o	70	34	10	1.2	1.5	Normal
21	18	95	1st	1740	o	55	43	18	1.8	0.9	
			3d	1670	o	55	40	17	2.1	0.9	
			6th	1580	o	55	55	21	1.5	1.0	
			10th	1530	o	50	34	11	1.2	0.7	
			13th	1470	o	50	29	14	1.4	0.5	
			18th	1240	..	..	40	23	1.4	0.9	Normal
17	11	75	1st	2450	++	60	32	7	1.3	0.5	
			3d	2400	o	60	35	9	1.6	0.6	
			8th	1900	o	60	75	46	1.7	0.5	Normal
Average							39	15	1.6	1.0	

condition also occurred in the vicinity of the internal elastic lamina of affected vessels.

This process was accompanied by a retention of nitrogenous products in the blood and the appearance of albumin in the urine, indicating serious interference with renal function.

The composite histological changes in the kidneys of the animals were strikingly different from the picture of any known pathological process occurring in man. Nevertheless, after decalcification, the lesions in the glomerular arterioles and the interlobular arteries did resemble the renal lesions associated with hypertension. Blood pressure readings were not taken because of technical difficulties.

Another unusual feature was the frequent occurrence of widespread tubular atrophy with very little demonstrable damage to the glomerular tufts. It is thought that perhaps this atrophy was secondary to vessel damage and to the tremendous thickening of the basement membranes of the tubules.

Collip *et al.* [13] has reported a rise in the non-protein nitrogen of the blood of dogs to as high as 219 mg. per 100 cc., occurring just before death as a result of large doses of parathyroid hormone. This substance, like irradiated ergosterol, causes a marked increase in blood calcium, with calcium deposition in various organs. Kidney lesions occur, as reported by Hueper [14] and Learner,[15] but the parenchyma is more seriously affected than are the blood vessels. Hueper describes, in dogs that received large doses of parathyroid extract, hemorrhages in the glomeruli, necrosis and calcification of the tubular epithelium, and "calcification of the membranæ propriae of the tubules and Bowman's capsules and of the elastic membranes of the arteries." Learner records similar changes in rabbits, but states that "it was in the lumen of the tubuli where the calcium was most to be seen."

It is noteworthy that the doses of irradiated ergosterol used in our experiments were many times greater, per unit of body weight, than those recommended for human use.

Summary

1. The administration of massive doses of irradiated ergosterol to rabbits caused marked histological changes in the kidneys. The chief changes were sclerosis and hyalinization of the vessel walls, and thickening of the basement membranes of the tubules and

glomerular capsules, accompanied in both by extensive subepithelial deposits of hyalin. There was abundant deposition of calcium in these localities. Pronounced atrophy of the tubular epithelium also occurred.

2. The kidney lesions were accompanied by the appearance of large amounts of albumin in the urine and by retention of nitrogenous products in the blood. The degree of nitrogen retention was in general proportional to the amount of kidney damage as evidenced by histological examination.

We wish to acknowledge our indebtedness to Dr. F. B. Mallory for helpful criticism and for the photomicrographs.

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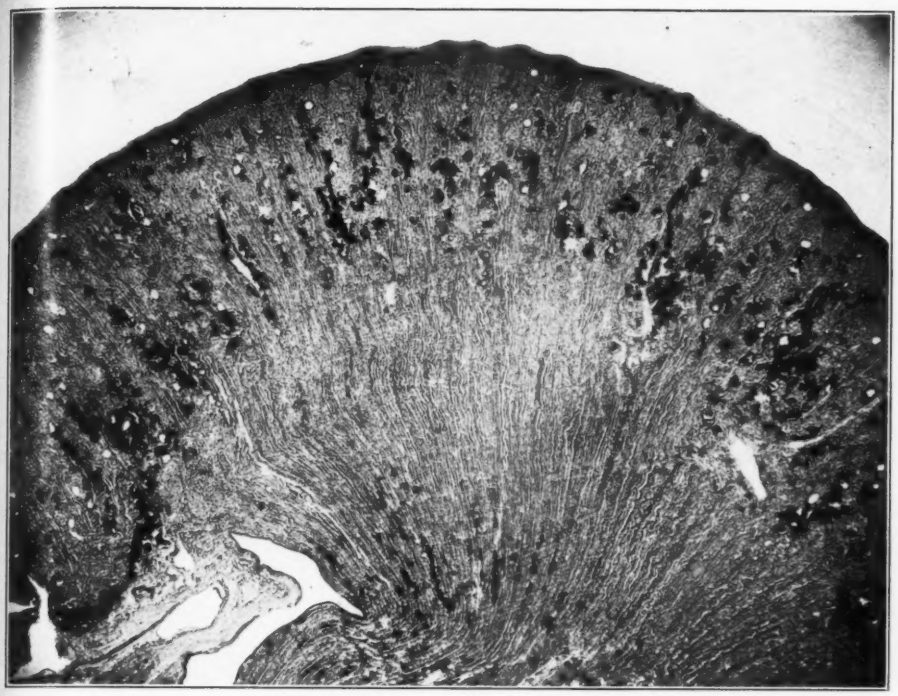
DESCRIPTION OF PLATES

PLATE 101

FIG. 1. Shows numerous dark staining deposits of calcium in the cortex and deep in the medulla. × 15.

FIG. 2. Four arterioles undergoing hyalinization, and narrowing. Also note hyaline deposit in a portion of the glomerular capsule. × 500.



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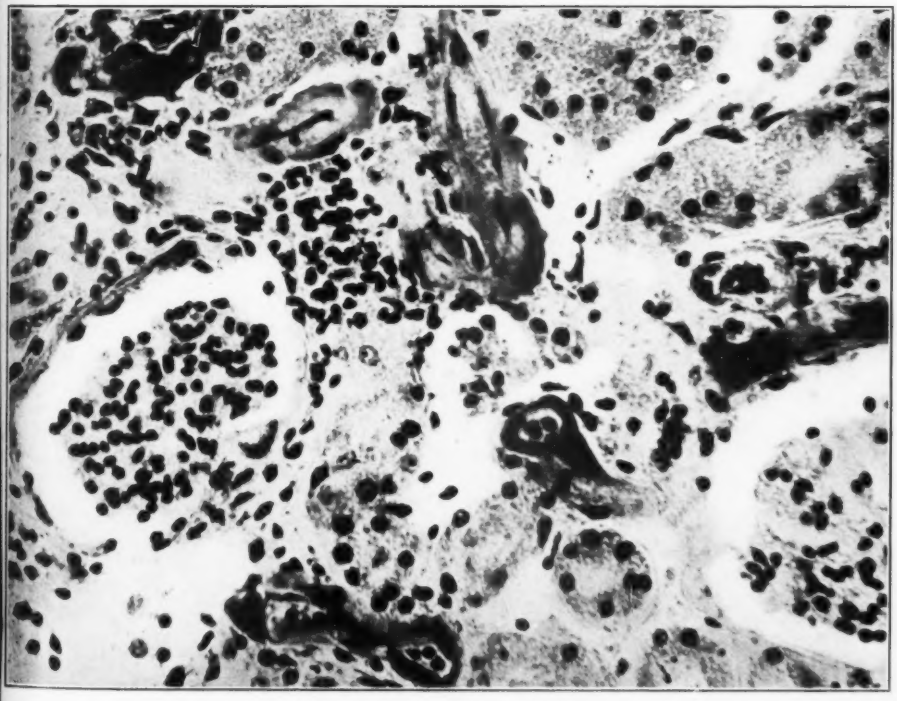


2

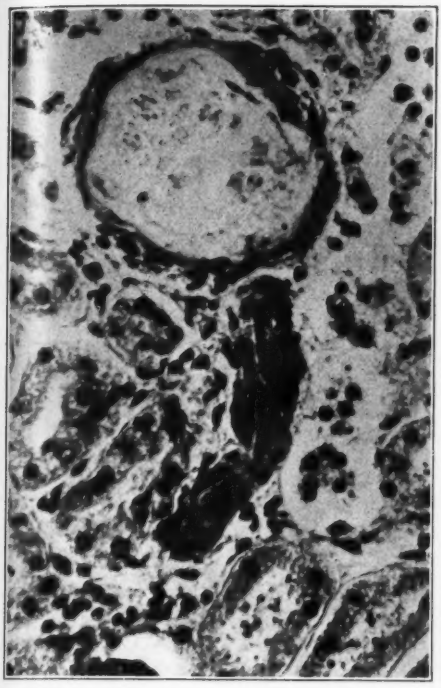
Spies and Glover Renal Lesions Produced by Irradiated Ergosterol

PLATE 102

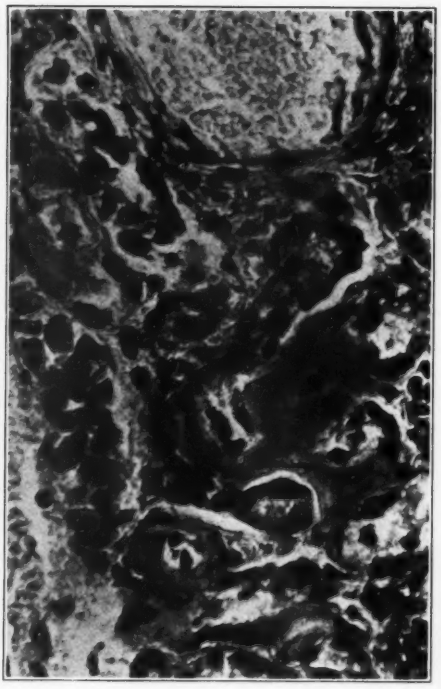
FIG. 3. Large interlobular artery with irregularly distributed subintimal deposits of hyalin. Adjacent arteriole, shown in longitudinal section, is undergoing marked hyalinization with reduction in caliber. × 500.

FIG. 4. Focal area of atrophied tubules containing thick deposits of hyalin. Note a portion of large artery is essentially negative. Compare these tubules with the more normal tubules in Fig. 2. × 500.

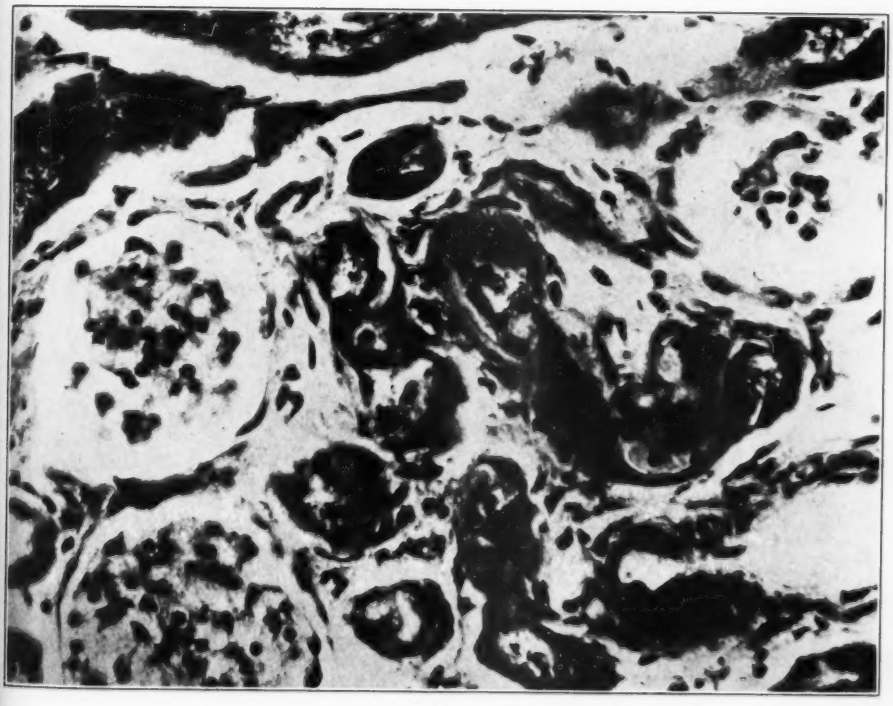
FIG. 5. Marked atrophy of the tubules with resulting approximation of the three glomeruli. Compare these atrophic tubules with the more normal tubules in Fig. 2. × 500.



3



4



5

Spies and Glover

Renal Lesions Produced by Irradiated Ergosterol